SIMULTANEOUS ESTIMATION OF FINATERIDE AND TAMSULOSIN HYDROCHLORIDE IN PHARMACEUTICAL DOSAGE FORMS BY UV SPECTROPHOTOMETRIC, RP-HPLC AND HPTLC METHODS.

A dissertation submitted to

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MASTER OF PHARMACY
IN
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By

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CERTIFICATE

This is to certify that this dissertation entitled "**Simultaneous Estimation Of Finateride And Tamsulosin Hydrochloride In Pharmaceutical Dosage Forms By UV Spectrophotometric, RP-HPLC And HPTLC Methods."** submitted **by** the candidate, **Mrs. M.S. Suganya**, for the award of degree in **Master of Pharmacy [Pharmaceutical Chemistry]**, is a bonafide record of the research work carried out by her under my guidance and supervision during her course of study at Madras Medical College, Chennai – 600 003, and that it has not previously formed the basis for the award of any degree or any other similar title and that it is an independent work done by her.

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LIST OF ABBREVIATIONS

S.No.	ABBREVIATION	
1.	FINA	Finasteride
2.	TAMS	Tamsulosin Hydrochloride
3.	EAC	Ethyl acetate
4.	CAN	Acetonitrile
5.	mg	milligram
6.	µg	microgram
7.	ml	millilitre
8.	µl	microlitre
9.	HPLC	High Performance Liquid Chromatography
10.	HPTLC	High Performance Thin Layer Chromatography
11.	UV	Ultra-Voilet Spectroscopy
12.	TLC	Thin Layer Chromatography
13.	LOD	Limit of Detection
14.	LOQ	Limit of Quantitation
15.	MM	milliMole
16.	%RSD	Relative Standard Deviation
17.	SE	Standard Error
18.	CI	Confidence Interval
19.	Std	Standard
20.	R_f	Retardation factor

INTRODUCTION

Analytical Chemistry, which is both theoretical and a practical science, is practiced in a large number of laboratories in many diverse ways. Methods of analysis are routinely developed, improved, validated, collaboratively studied and applied.

Quality assurance plays a central role in determining the safety and efficacy of medicines. High specific and sensitive analytical techniques hold the key to the design, development, standardization and quality control of medicinal products. Quality level of any analytical work in a quality control laboratory depends on expertise of the analyst, most appropriate analytical procedure and overall performance of the analytical instruments. The main task in the pharmaceutical analyst is therefore to provide reliable analytical data rapidly and as accurate as required, repeatedly (or daily) at low cost and on a wide range of different samples, substances and materials.

The analytical chemist is often confronted with the difficulty of selecting the most suitable method for the required determination.

Modern analytical chemistry has most diverse methods and techniques of analysis for quantitative determination. Their classification is based on different principles.

They are broadly classified in to

- ❖ Chemical methods of analysis
- ❖ Physical methods of analysis

Chemical Methods

It involves the measurement of the mass of the substance or the volume of the reacting solutions. Eg : Gravimetric and volumetric methods

Physical Methods

It involves the measurement of physical parameters of the system, which is related to the amount of component being determined.

Eg : Spectral methods and electrochemical methods.

Instrumental or physico chemical methods are based on the theory or relationship between the content and the corresponding physicochemical and physical properties of the chemical system being analyzed.

The five important techniques that are indispensable to analytical chemistry are

- ❖ Gravimetric Analysis
- ❖ Volumetric Analysis
- ❖ Optical Methods
- ❖ Electrical Methods
- ❖ Separation Methods

SPECTROSCOPY

It is the measurement and interpretation of electromagnetic radiations absorbed or emitted when the molecules or atoms or ions of the sample undergo transition from one energy state (Ground state) to another (Excited state).

It is of two types.

1. Absorption Spectroscopy

Where absorption of electro magnetic radiation (EMR) takes place.

(Eg) UV Spectroscopy, Colorimetry, IR Spectroscopy, etc.,

2. Emission Spectroscopy

Where emission of radiation is being studied. (Eg) Flame photometry flourimetry.

CHROMATOGRAPHY

Chromatography is a powerful separation method, which finds application in all branches of science. Chromatography was invented and named by the Russian botanist Mikhail Tswett at the beginning of the twentieth century. He employed this technique to separate plant pigments such as chlorophylls, Xanthophylls using glass columns packed with finely divided calcium carbonate. The separated species appeared as coloured bands on the

column, which accounted for the Greek name, he chose for the method as "chroma" meaning "colour" and "graphein" meaning to "write".[1]

In all chromatographic separations the sample is transported in a mobile phase, which may be a gas, liquid, or a supercritical fluid. This mobile phase is then forced through an immiscible stationary phase, which is fixed in place in a column or on a solid surface. The two phases are chosen, so that the components of the sample distribute themselves between the mobile and stationary phase to varying degrees. Those components that are strongly retained by the stationary phase, move only slowly with the flow of mobile phase. In contrast, components that are weakly held by the stationary phase travel rapidly. As a consequence of these differences in mobility, sample components separate into discrete bands as zones, that can be analysed qualitatively and / or quantitatiavely. [1]

Chromatographic methods can be classified according to the nature of the stationary and mobile phases.

The different types of chromatography are

1. Adsorption chromatography
2. Partition chromatography
3. Ion exchange chromatography
4. Size exclusion or gel permeation chromatography.

The modern instrumental techniques of GLC, HPTLC and HPLC provide excellent separation and allow accurate assay of very low concentrations of wide variety of substance in complex mixtures.

VALIDATION[2,3,4]

Validation of an analytical method is the process that establishes, by laboratory studies, that the performance characteristics of the method meet the requirements for the intended analytical applications[2].

REASONS FOR VALIDATION

- Enables scientists to communicate scientifically and effectively on technical matters.
- Setting standards of evaluation procedures for checking complaints and taking remedial measures
- Retrospective validation is useful for trend comparison of results compliance to cGMP/GLP.
- Closer interaction with pharmacopoeia harmonization particularly in respect to determination of impurities and their limits.
- For taking appropriate action, in case of non – compliance.
- To provide high degree of confidence that the same level of quality is consistently built into each unit of finished product from batch to batch.

- Economic: The consistency and reliability of validated analytical procedure is to produce a quality product with all the quality attributes, thus providing indirect cost saving from reduced testing or re testing and elimination of product rejection.

- As quality control process is not static, some form of validation / Verification should continue till the validated process is in use.

Typical Analytical Characteristics used in Method Validation[2]

- Accuracy
- Precision
- Specificity
- Detection limit
- Quantitation limit
- Linearity
- Range
- Ruggedness
- Robustness
- System suitability

Accuracy

The accuracy of an analytical procedure expresses the closeness of the test results obtained by that method to the true value (a conventional true value or an accepted reference).

Accuracy is calculated as the percentage of recovery by the assay of the known added amount of analyte in the sample, or as the difference between the mean and the accepted true value, together with confidence intervals.

The ICH documents recommend that accuracy be assessed using a minimum of nine determinations over a minimum of three concentration level covering the specified range (i.e., three concentrations and three replicates of each concentration.)

Precision

The precision of an analytical procedure is the degree of agreement among the individual test results when the method is applied repeatedly to multiple samplings of a homogenous sample under the prescribed conditions. Precision of an analytical procedure is usually expressed as the standard deviation or relative standard deviation (coefficient of variation) of a series of measurements. Precision may be a measure of either the degree of reproducibility or repeatability of the analytical method under operating conditions.

- **Repeatability** refers to the use of the analytical procedure within the laboratory over a short period of time using the same analyst with same equipment.

- **Reproducibility** refers to the use of analytical procedure in different laboratories as in a collaborative study.

- **Intermediate Precision** expresses within laboratory variation, as on different days or with different analysts or equipment within the same laboratory.

ICH documents recommends that repeatability should be assessed using a minimum of nine determinations covering the specified range for the procedure (i.e., three concentration and three replicates of each concentration) or a minimum of six determination at 100% of the test concentration.

Specificity

Specificity is the ability to assess unequivocally the analyte in the presence of components, which may be expected to be present. Lack of specificity of an individual analytical procedure may be compensated by other supporting analytical procedures.

Detection limit

The detection limit of an individual analytical procedure is the lowest amount of analyte in a sample, which can be detected but not necessarily quantitated as an exact value.

Based on the standard deviation of the response and the slope, the detection

Limit (LOD) may be expressed as

$$DL = \frac{3.3\ \sigma}{S}$$

Where,

σ = standard deviation of the response

S= slope of the calibration curve (of the analyte)

Quantitation limit

The quantitation limit of an analytical procedure is the lowest amount of analyte in a sample, which can be quantitatively determined with suitable precision, accuracy and reliability by the proposed method.

Based on the standard deviation of the response and the slope, quantitation limit may be expressed as

$$LOQ = \frac{10\ \sigma}{S}$$

Where,

σ = standard deviation of the response

S = slope of the calibration curve (of the analyte)

Linearity

Linearity of an analytical method is its ability to elicit the results that are directly or by a well-defined mathematical transformation proportional to the concentration of analyte in sample within a given a range.

Range

Range of an analytical procedure is the interval between the upper and lower levels of analyte (including these concentrations) that has been demonstrated to be determined within a suitable level of precision, accuracy and linearity using the method as written.

Ruggedness

The ruggedness of an analytical method is the degree of reproducibility of test results obtained by the analysis of the same samples under variety of conditions, such as different laboratories, analysts, instruments, lots of reagents, elapsed times, assay temperatures or days. Intermediate precision can be considered as ruggedness.

Robustness

The robustness of an analytical method is a measure of its capacity to remain unaffected by small but deliberate variations in method parameters and provides an indication of its reliability during normal usage.

System suitability[3]

System suitability tests, plays a fundamental role in all the analytical procedures, are based on the concept that the equipment, electronics, analytical operations and samples to be analyzed constitute an integral system that can be evaluated as such.

The system suitability tests are an integral part of gas and liquid chromatographic methods.

- **Retention time (R_t)** is the time of emergence of the maximum of a component after injection.

- **Symmetry factor (or) tailing factor (T)** is a measure of peak symmetry, is unity for perfectly symmetrical peaks. Its value increases as tailing become more pronounced. As peak asymmetry increases, integration and hence precison, becomes less reliable.

$$T = \frac{W_{0.05}}{2f}$$

The assessment of peak shape is in terms of symmetry factor.

$W_{0.05}$ = width of the peak at 5% height.

f = distance from the peak maximum to the leading edge of the peak, when measured at 5% peak height (from the baseline).

- **Number of theoretical plates (N)** is measure of column efficiency. If the number of theoretical plate is high, the column is said to be highly efficient and vice versa. It is a measure of sharpness, which is important for the detection of trace elements.

$$N = 5.54 \left[\frac{t}{w_h/_2} \right]^2$$

The assessment of performance of efficient of a column is in terms of the number of theoretical plates.

- **Resolution** is a measure of relative separation of two peaks. Resolution is defined as the distance between the two band centres divided by the average width of the peaks determined at the bases of peaks.

$$R = \left[\frac{2(t_2-t_1)}{W_2+W_1} \right]$$

Where,

t_2, t_1 are retension times of first component and 2nd component respectively.

W_2 , W_1 are width of peak of first component and 2nd component, eluted.

Statistical parameters

Statistics consist of a set of methods and rules for organizing and interpreting observations.

The precision or reproducibility of the analytical method was determined by repeating the analysis six times and the following statistical parameters were calculated.

The Formulas are[4]

$$\text{Standard Deviation} = \frac{\Sigma(x - \bar{x})^2}{n - 1}$$

$$\text{R.S.D (\%)} = \frac{\text{S.D}}{\bar{x}} \times 100$$

$$\text{S.E} = \frac{\text{S.D}}{\sqrt{n}}$$

Confidence Interval / Limits (CI):

The confidence limits describe the range within which we expect with given confidence the true value to lie.

$$CL = \bar{x} \pm 1.96\,\sigma / \sqrt{n}$$

Where,

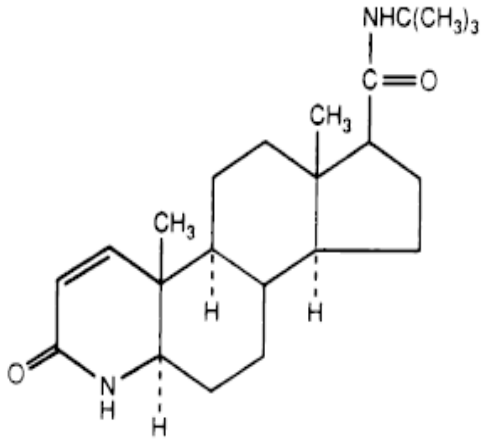
Σ	=	Sum of observations
$\bar{x}$	=	Mean or arithmetic average ($\Sigma x / n$)
x	=	Individual observed value
$x - \bar{x}$	=	Deviation of a value from the mean
n	=	Number of observations
S.D	=	Standard deviation

DRUG PROFILE

I. FINASTERIDE[5]

Finasteride is 4-azaandrost-1-ene-17-carboxamide,*N*-(1,1-dimethylethyl) -3-oxo-,(-,(5α,17β)-. The empirical formula of finasteride is **$C_{23}H_{36}N_2O_2$** and its molecular weight is 372.55.

Its structural formula is:



Description

Finasteride is a white crystalline powder with a melting point near 250°C.

Solubility

It is freely soluble in chloroform and in lower alcohol solvents but is practically insoluble in water.

Mechanism of Action

Finasteride, a synthetic 4-azasteroid compound, is a competitive and specific inhibitor of steroid Type II 5α-reductase, an intracellular enzyme that converts the androgen testosterone into 5α-dihydrotestosterone (DHT). Two distinct isozymes are found in mice, rats, monkeys, and humans: Type I and II.

Indications and usage

Finasteride is indicated for the treatment of male pattern hair loss (androgenetic alopecia) in **MEN ONLY**. Safety and efficacy were demonstrated in men between 18 to 41 years of age with mild to moderate hair loss of the vertex and anterior mid-scalp area. Finasteride is not indicated in women and children

Contraindications

Finasteride use is contraindicated in women when they are or may potentially be pregnant, because of the ability of Type II 5α-reductase inhibitors to inhibit the conversion of testosterone to DHT, finasteride may cause abnormalities of the external genitalia of a male fetus of a pregnant woman who receives finasteride.

Warnings

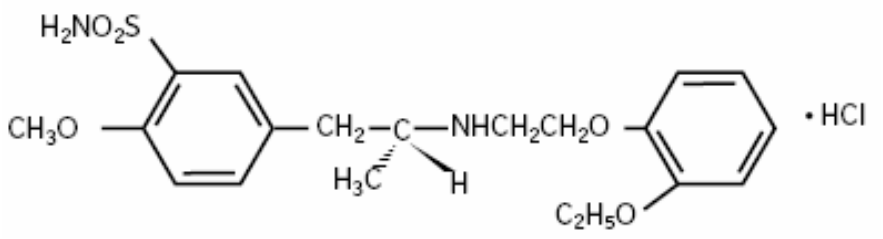
Finsteride is not indicated for use in pediatric patients or women.

Exposure of women - risk to male fetus

Women should not handle crushed or broken Finsteride tablets when they are pregnant or may potentially be pregnant because of the possibility of absorption of finasteride and the subsequent potential risk to a male fetus. Fiinsteride tablets are coated and will prevent contact with the active ingredient during normal handling, provided that the tablets have not been broken or crushed.

II. TAMSULOSIN HYDROCHLORIDE[6]

Tamsulosin HCl is (-)-(R)-5-[2-[[2-(*o*-Ethoxyphenoxy) ethyl]amino] propyl]-2-methoxybenzenesulfonamide, monohydrochloride. The empirical formula of tamsulosin HCl is **$C_{20}H_{28}N_2O_5S.HCl$**. The molecular weight of tamsulosin HCl is 444.98. Its structural formula is:



Description

Tamsulosin HCl occurs as white crystals that melt with decomposition at approximately 230°C.

Solubility

It is sparingly soluble in water and in methanol, slightly soluble in glacial acetic acid and in ethanol, and practically insoluble in ether.

Mechanism of action

Tamsulosin hydrochloride is an antagonist of alpha$_{1A}$ adrenoceptors in the prostate. The symptoms associated with benign prostatic hyperplasia (BPH) are related to bladder outlet obstruction, which is comprised of two underlying components: static and dynamic.

The static component is related to an increase in prostate size caused, in part, by a proliferation of smooth muscle cells in the prostatic stroma. The dynamic component is a function of an increase in smooth muscle tone in the prostate and bladder neck leading to constriction of the bladder outlet.

Tamsulosin hydrochloride capsules are not intended for use as an antihypertensive drug.

Indications and usage

Tamsulosin hydrochloride capsules are indicated for the treatment of the signs and symptoms of benign prostatic hyperplasia (BPH). Tamsulosin Hydrochloride capsules are not indicated for the treatment of hypertension.

Contraindications

Tamsulosin hydrochloride capsules are contraindicated in patients known to be hypersensitive to tamsulosin hydrochloride.

Warnings

The signs and symptoms of orthostasis (postural hypotension, dizziness and vertigo) and potential risk of syncope (as with other alpha-adrenergic blocking agents) were detected more frequently in Tamsulosin hydrochloride capsule treated patients.

Precautions

- ***General***

1. *Carcinoma of the prostate:* Carcinoma of the prostate and BPH cause many of the same symptoms. These two diseases frequently co-exist. Patients should be evaluated prior to the start of Tamsulosin hydrochloride capsules therapy to rule out the presence of carcinoma of the prostate.

2. *Intraoperative Floppy Iris Syndrome (IFIS) has been observed during cataract* surgery in some patients treated with alpha-1 blockers, including Tamsulosin hydrochloride capsules.

3. *Drug-Drug Interactions with Tamsulosin Hydrochloride capsules:* - should NOT be used in combination with other alpha-adrenergic blocking agents.

- should be used with caution in combination with cimetidine, particularly at doses higher than 0.4 mg.

- Caution should be exercised when concomitantly administered with warfarin.

- Caution should be exercised while operating machinery or driving due to possibiliity of postural hypotension such as dizziness as side effect of the drug.

- ***Special precautions***

1. Pregnancy

Teratogenic Effects**,** *Pregnancy Category B.*

Tamsulosin Hydrochloride capsules are not indicated for use in women.

2. Nursing Mothers and Pediatric Use

Tamsulosin Hydrochloride capsules are not indicated for use in women and in pediatric populations.

OBJECTIVE OF STUDY

In view of the literature cited, for the simultaneous quantification of Finasteride and Tamsulosin hydrochloride in pharmaceutical dosage forms, there is no documentary evidence available. At the outset, it was aimed to develop a rapid, sensitive, precise and accurate method for the pharmaceutical dosage forms.

The objective of the study is as follows:

- Development of rapid, sensitive and accurate reverse phase RP-HPLC method for simultaneous estimation of Finasteride & Tamsulosin hydrochloride in pharmaceutical dosage forms.

- Development of rapid, sensitive and accurate High Performance Thin Layer Chromatographic method for simultaneous estimation of Finasteride & Tamsulosin hydrochloride in pharmaceutical dosage forms.

- Development of UV Spectrophotometric method for simultaneous estimation of Finasteride & Tamsulosin hydrochloride in pharmaceutical dosage forms by Q-absorbance ratio method.

REVIEW OF LITERATURE

Carlin-JR et al[7] (1988) described a sensitive and selective HPLC method with UV detection for the quantitative determination of MK-906 (N-(2-methyl-2-propyl)-3-oxo-4-aza-5alpha-androst-1-ene-17beta-carboxamide; finasteride) in human plasma. Human plasma levels were reported for the drug following single dose oral administration of 50, 200 and 400 mg; urinary excretion data were reported for a single volunteer given 400 mg of drug.

Soeishi-Y et al[8] (1990) reported a simple, precise and sensitive HPLC method with fluorescence detection for the determination of Tamsulosin hydrochloride in human plasma. The utility of the method was demonstrated by monitoring the plasma concentration of unchanged drug after oral administration of 0.2 mg of the drug to 12 healthy volunteers.

Constanzer-ML et al[9] (1991) developed a simple, specific and sensitive HPLC method with UV detection is described for the quantitation of finasteride in human plasma. The method was used to determine the plasma drug level in a human subject following oral administration with 5 mg finasteride.

Ryan-JA et al[10] (1991) published a mid-infrared diffuse reflectance spectroscopic method for the rapid verification of identity and content of drugs in dosage forms. The method was applied to the analysis of simvastatin, enalapril maleate, lovastatin and finasteride in dosage forms.

Hiroshi Matsushima et al[11] (1997) reported a highly sensitive method for the determination of tamsulosin hydrochloride, in human plasma dialysate,

plasma and urine by using liquid chromatography–electrospray tandem mass spectrometry (LC–MS–MS). Plasma dialysate, plasma and urine samples were extracted by brief liquid-phase extraction and analyzed using an HPLC system coupled to a mass spectrometer via an electrospray ionization interface. Selected reaction monitoring was used for the detection of tamsulosin and its internal standard. This method was validated in the concentration range 10–1000 pg/ml in plasma dialysate, 0.5–50 ng/ml in plasma, and 1–100 ng/ml in urine with sufficient specificity, accuracy and precision.

Syed-AA et al[12](2001) reported, LC determination of finasteride and its application to storage stability studies.

Ilango-K et al[13] (2002) published a simple and sensitive method was developed for the estimation of finasteride in tablet formulations. The method was based on the reaction of the drug with 0.2 % w/v 3-methyl-2-benzthiazolinone hydrazone (MBTH) reagent in presence of 7% w/v ferric chloride solution to yield, a green colour, which had the characteristic light absorption in the visible region with an absorption maximum at 446 nm. The chromogen formed was stable for 45 min. Beer's law is obeyed in the concentration range of 2 to 10 mug/ml. The reproducibility of the method is 98.8%. The proposed method was precise, accurate and reproducible and was extended to the analysis of finasteride in the tablet formulations.

Loi-AL et al[14] (2002) reported a retrospective chart review to facilitate the conversion of tamsulosin to formulary alpha-blockers in the treatment of benign prostatic hypertrophy;

Li-XY et-al[15] (2003) developed the determination of finasteride in human plasma by HPLC-MS.

HPLC-MS assay for determination of finasteride in human plasma and to investigate the bioequivalence in healthy volunteers was observed. After alkalization with NaOH, plasma was extracted with ethyl acetate and separated using a C<INF>18</INF> column with a mobile phase of methanol-water (85:15). LC-ESI-MS was performed in the selected ion monitoring (SIM) mode using target ions at m/z 395 for finasteride and m/z 407 for IS. Fragmentor voltage was 120 V. Randomized crossover design was performed in 20 healthy volunteers. In the study periods, a single 10 mg dose of each tablet was administered to each volunteer. Result: Calibration curves were linear over the range 1-200mug/L. Limit of determination for finasteride in plasma was 0.05mug/L. Recovery of finasteride from plasma was in the range of 85.9%-98.7%. Results of variance analysis and two one-side t-test showed that there was no significant difference between two formulations in the AUC and C<INF>max</INF>. Conclusion made that the assay was sensitive, accurate and convenient. Two formulations were bioequivalent.

Zhang-ZF et al[16] (2004) developed A method for the chiral separation of tamsulosin isomers by high performance liquid chromatography using cellulose tris (3,5-dimethylphenylcarbamate) as a chiral stationary phase is described. The precision, and calibration of the method is discussed.

A high-performance liquid chromatographic (HPLC) method was developed for the chiral separation of an antagonist of alpha1A adrenoceptors,

tamsulosin and its *S*-isomer. Baseline separation of the isomers was achieved within 35 min on a CHIRALCEL OD-RH column with a binary solvent mixture of 50 mmol l^{-1} KPF_6–acetonitrile (v/v (70:30), pH 5.0) as the optimized mobile phase. The detection limits and quantification limits of both *R*-isomer and *S*-isomer were 0.11 and 0.44 ng, respectively. The R.S.D. values of peak-area for the two isomer were 0.42% (of peak-height: 0.77%) for *R*-isomer and 0.64% (of peak-height:0.92%) for *S*-isomer (*n*=5).

***Maier -V et al*[17] (2005)** devised a method for Chiral separation of tamsulosin by capillary electrophoresis.

Enantiomers of (±) 5-[2 (*R*,*S*)-{[2-(*o*-ethoxyphenoxy) ethyl] amino} propyl]-2-methoxy-benzenesulfonamide (tamsulosin, drug frequently used in the treatment of prostate diseases) were separated by capillary electrophoresis (CE). An acidic background electrolyte (BGE) with sulfated-β-cyclodextrin (S-β-CD) was used to create a chiral separation environment. Baseline separation of the isomers was achieved during 5 min using cathodic electro-osmotic flow (EOF) (countercurrent mode). The quantification limits were 5.3×10^{-6} mol l^{-1} for *R*-isomer and 5.7×10^{-6} mol l^{-1} for *S*-isomer. The R.S.D. values of peak area were 0.54% for *R*-isomer and 0.75% for *S*-isomer. The results achieved enable determination of 0.5% of optical impurity.

***Hulya Demir et al*[18] (2006)** presented a liquid chromatography method and its analytical method validation for the determination of finasteride in the tablet form.

A quantitative method for finasteride by liquid chromatography (LC) with UV detector, was validated for its new tablet form Dilaprost®. Analysis was performed using Nova Pak C_{18} column at 60 °C. Detection was carried out at a wavelength of 210 nm. The best separation for finasteride peak was achieved by isocratic elution with the mobile phase water/acetonitrile/ tetrahydrofuran (80/10/10, v/v/v) and flow rate of 2 mL min^{-1}. The sample volume injected into liquid chromatography system was 15 µL. Analytical method validation tests were performed.

K. Basavaiah et al[19] (2006) presented three sensitive spectrophotometric – bromatometric assay methods for the determination of finasteride in bulk and in tablets. The methods rely on the use of bromate-bromide reagent and three dyes namely, methyl orange, indigocarmine and thymol blue as reagents. They involve the addition of a measured excess of bromate-bromide reagent to finasteride in acid medium, and after the bromination reaction is judged to be complete, the unreacted bromine is determined by reacting with a fixed amount of either methylorange and measuring the absorbance at 520 nm (method A) or indigocarmine and measuring the absorbance at 610 nm (method B) or thymol blue and measuring the absorbance at 550 nm (method C).

Pekka Keski-Rahkonen et al[20] (2006) developed a simple, sensitive and selective LC–MS/MS method for the determination of tamsulosin in human aqueous humor and serum to study the recently reported eye-related adverse effects of this α_1-blocker drug. Aqueous humor samples were analyzed by direct injection, after addition of the internal standard, labetalol. Liquid–liquid extraction with ethyl acetate was used for serum sample preparation. The

chromatographic separation was performed on a reversed phase column by gradient elution with acetonitrile −0.1% formic acid at a flow-rate of 0.2 ml/min.

USP 29[21] exhibits a RP-HPLC assay method for Finasteride Tablets as an offical monograph. The method was established with L1 columns, Acetonitrile: Phosphoric acid (2.5mM) in 1:1 ratio as mobile phase and UV detector wavelength as 240nm for estimation of the drug in tablets.

MATERIALS AND METHODS

EXPERIMENTAL DETAILS

The following methods were developed for the Simultaneous estimation of Finasteride and Tamsulosin Hydrochloride in Pharmaceutical dosage forms.

- Reverse Phase High Performance Liquid Chromatography
- High Performance Thin Layer Chromatography.
- Q-absorbance ratio method by UV-Visible Spectrophotometry

1. Reverse Phase High Performance Liquid Chromatography

High Performance Liquid Chromatography[22, 23] is the most widely used of all the analytical separation techniques, owing to its sensitivity; its ready adaptability to accurate quantitative determinations; its suitability for separating non-volatile species or thermally fragile ones and its widespread applicability to substance like aminoacids, proteins, carbohydrates, drugs, terpenoids, antibiotics, steroids that are of prime interest to many field of science. The technique of HPLC was developed in the late 1960s and early 1970s from the theoretical knowledge of already established principles of earlier chromatographic techniques. The technique is based on the modes of separation i.e., adsorption, partition (including reverse phase partition), ion-exchange and gel permeation, but it differs from column chromatography in that the mobile phase is pumped through the packed column under high pressure. The principal advantages of HPLC compared to classical (gravity feed) column chromatography are improved resolution of the separated substances, faster separation times and the increased accuracy, precision and sensitivity with which the separated substances may be quantified.

The apparatus of HPLC includes pump, injection systems, column, detectors, recorder and data output system.

HPLC is one of the most versatile instrument used in the field of pharmaceutical analysis. It provides the following features.

1. High resolving power
2. Speedy separation
3. Continuous monitoring of the column effluent
4. Accurate quantitative measurement
5. Repetitive and reproducible analysis using the same column
6. Automation of the analytical procedure and data handling

In HPLC the analyst has a wide choice of chromatographic separation methodologies from normal to Reverse Phase and a whole range of mobile phases using isocratic or gradient elution techniques.

Various detectors available for HPLC are electrochemical detectors, refractive index detectors, fluorescence detectors, radiochemical detectors, mass-sensitive detectors and Ultra-violet (UV) detectors.

Reverse -Phase High Performance Liquid Chromatography

Reversed-phase chromatography refers to the use of a polar eluent with a non-polar stationary phase in contrast to normal-phase chromatography, where a polar stationary phase is employed with a non-polar mobile phase.

In reverse-phase liquid chromatography the stationary phase is prepared by chemically bonding a relatively non-polar group on to the stationary phase support. The most frequent non-polar group on to the stationary support is octadecylsilane (ODS or C18), which gives a highly lipophilic stationary phase.

Less lipophilic stationary phases are produced when octylsilance (C8, C2, phenyl or cyanoprophyl) – bonded phases are used. The shorter silanyl chain bonded phases (C2 and C8) are often most appropriate when highly lipophilic solutes are to be separated which would be highly retained on C18 column.

Reversed-Phase Mobile phases

The power of HPLC in terms of being able to resolve many compounds is mainly due to the diversity of mobile phases or mobile solvents available. The mobile phases in reversed phase HPLC generally comprises of water and a less polar organic solvents, e.g. methanol or acetonitrile. The rate of elution of the components is controlled by the polarity of the organic solvents and its proportion in the mobile phase. Further selectivity in the separation of ionisable substances may be obtained by altering the pH of the mobile phase to cause ion suppression. The water should be of high quality either distilled or deminieralised. When inorganic salts and ionic surfactants are used, the mobile phase should be filtered before use since these additives frequently contain a significant amount of water insoluble contaminants that may damage the column. Reverse-phase mobile phases are generally non-inflammable due to high water content. Degassing is quite important with reversed-phase mobile phases.

A. INSTRUMENTS

High performance Liquid Chromatograph

- Shimadzu Prominence separation module equipped with pumps LC-10AT
- UV-Visible detector SPD 10A
- Phenomenex column. Luna 5µ C18 – Spherical. Size: 250 x 4.60mm
- Rheodyne valve injector with 20µl fixed loop

Hamilton syringe (25µl syringe)

Ultipor N66 (Nylon 6.6 membrane filter)

pH meter – Digisun electronics

Sonicator – Enertech electronics

Electronic Balance - Mettler AB 54.

B. REAGENTS AND CHEMICALS:

Acetonitrile HPLC grade

Water HPLC grade

Disodium hydrogen orthophosphate anhydrous AR grade

Orthophosphoric acid AR grade

Finasteride USP – Preanalysed raw material

Tamsulosin Hydrochloride - preanalysed raw material

Market sample of formulation from a local pharmacy

C. OPTIMIZATION OF CHROMATOGRAPHIC CONDITIONS:

The chromatographic conditions were optimized using C18 column and mobile phase was developed with acetonitrile and buffer (adjusted to pH 3) in different ratios.

The retention time of Finasteride and Tamsulosin Hydrochloride were determined by injecting individual standard drug solutions separately. Then the mixed standard solutions of Finasteride and Tamsulosin Hydrochloride were prepared with the concentration in the ratio of 12.5:1 respectively. This stock solution was diluted to 100mcg and 8mcg of Finasteride and Tamsulosin Hydrochloride and then injected for all the trials made. In all the trials, the respective mobile phase are used as diluent. UV λmax of the individual drugs were determined at 210 nm for Finasteride and 225nm for Tamsulosin Hydrochloride. UV – visible Detector was used to detect the signals.

Trial No.	**Mobile phase**	**λ (nm)**	**Flow Rate (ml/min)**	**Observation**
1.	ACN: Buffer (75:25 v/v)	220	1.0	The Capacity factor of Tamsulosin Hydrochloride is 0.35. Finasteride peak was not significant.
2.	ACN: Buffer (75:25 v/v)	230	0.75	The retension time of Tamsulosin was 2.0 minutes. The Finasteride peak had secondary peak near by.

Trial No.	Mobile phase	λ (nm)	Flow Rate (ml/min)	Observation
3.	ACN: Buffer (50:50 v/v)	230	0.75	The retension time of Tamsulosin was 2.05 mins. The retension time of Finasteride was extended to 8.79 minutes.
4.	ACN: Buffer (60:40 v/v)	230	0.75	The retension time of Tamsulosin was 2.69 mins. The retension time of Finasteride was extended to 7.79 minutes.
5.	ACN: Buffer (60:40 v/v)	240	0.75	Tamsulosin peak was not prominent.
6.	ACN: Buffer (60:40 v/v)	230	0.80	The retension time of Tamsulosin was 2.65 mins. The retension time of Finasteride was extended to 7.11 minutes.

D. OPTIMISED CHROMATOGRAPHIC CONDITIONS

Stationary Phase: C18 (250 x 4.6mm, 5µ) Phenomenex

Mobile phase: Acetonitrile : buffer adjusted to pH 3 (60:40 v/v)

Operation mode: Isocratic

Wavelength of UV detector: 230nm

Flow rate: 0.8ml/min

Diluent: Mobile phase

Injection volume: 20µl

Temperature: Ambient

Run time: 10 minutes

E. PREPARATION OF MOBILE PHASE

Preparation of Buffer

A quantity of 0.8875g (0.05mM) of disodium hydrogen orthophosphate anhydrous was dissolved in water and made up to 125ml with water. The pH was adjusted to 3.0 using Orthophosphoric acid and filtered through membrane filter applying vacuum. The mobile phase is prepared by mixing Acetonitrile and the buffer in the ratio of 60:40.

F. EXPERIMENTAL

The diluent was injected first to determine the absence of any interference with the baseline. The retention time of individual drugs are determined. Then the combination of drugs was injected in different concentrations and the chromatogram was developed under the optimized chromatographic condition.

Preparation of standard solution

About 125mg of Finasteride and 10mg of Tamsulosin Hydrochloride was weighed accurately and transferred into 25ml volumetric flask, dissolved

and diluted to volume with the mobile phase (ACN: Buffer – 60:40) to get the stock solution. From this serial dilutions were made to obtain a final concentration ranging from 50 – 150mcg/ml of Finasteride and 4-12mcg/ml of Tamsulosin Hydrochloride. Linearity was established in this concentration range (50 – 150%) and calibration curve was constructed, by injecting 20µl of the standard solutions. Linearity data are presented in Table No.1(a), 1(b) and the graphs are presented as Graph No.1(a), 1(b), 1(c) and 1(d).

Market Sample Analysis

The content of twenty capsules were accurately weighed and powdered. The powder equivalent to 12.5mg of Finasteride and 1mg of Tamsulosin Hydrochloride was accurately weighed and transferred into a 50ml volumetric flask. The content was dissolved in 25ml of mobile phase and subjected to sonication for 20 minutes. Then the resulting solution was made up to the volume with the mobile phase. This solution was filtered through a nylon membrane filter and first few ml of filterate was rejected. It was then diluted to yield a final solution, with the concentration of 100mcg/ml of Finasteride and 8 mcg of Tamsulosin Hydrochloride, which is used for further estimations.

The amount of drug present per capsule was calculated by adopting the following formula.

$$= \frac{\text{Area of Sample x Standard dilution x Average weight x Potency}}{\text{Area of Standard x Sample dilution x 100}}$$

The assay results are presented in the Table No.1(c)

Recovery Studies

Recovery studies were carried out by addition of a known quantity of the standard drug to the pre-analyzed samples (50, 100 and 150% of target level) and the whole contents were reanalyzed by the proposed method.

Percentage recovery was calculated by using the following formula:

$$= \frac{\text{Amt. found in solution} - \text{Actual amt. of preanalysed sample}}{\text{Amount of standard added}} \times 100$$

The data are presented in Table No.1(e) and 1(f).

METHOD VALIDATION

1. Specificity

The Specificity is the ability to assess unequivocally the analyte in the presence of components that may be expected to be present, such as impurities, degradation products and matrix components. This definition implies the identification tests, purity test and assays. The drugs, Finasteride and Tamsulosin have specific retension time in the optimised chromatographic conditions.

2. Linearity and Range

The linearity and range of the analytical method is determined from the calibration curve and mathematical transformations proportional to the

concentration of the analyte. The prepared standard stock solution is diluted to the concentration ranging from 50 to 150% (of 100% target level) of finasteride and Tamsulosin Hydrochloride. The data are presented in the Table No.1(a) and 1(b).

3. Precision (Method Precision, Intermediate Precision (Ruggedness))

Precision was evaluated by quantifying homogenous sample in six times for both intra-assay and inter-assay precision equivalent to 100µg/ml of Finasteride and 8µg/ml of Tamsulosin hydrochloride.

The amount of drug present per capsule was calculated by adopting the following formula.

$$= \frac{\text{Area of Sample x Standard dilution x Average weight x Potency}}{\text{Area of Standard x Sample dilution x 100}}$$

The precision data are presented in the Table No.1(d)

4. Accuracy (Recovery)

To ensure the accuracy of the method, recovery studies were carried out by addition of a known quantity of the standard drug to the pre-analyzed samples (50, 100 and 150% of target level) and the whole contents were reanalyzed by the proposed method.

Percentage recovery was calculated by using the following formula:

$$= \frac{\text{Amt. found in solution} - \text{Actual amt. of preanalysed sample}}{\text{Amount of standard added}} \times 100$$

The data on recovery studies are presented in Table no.1(e) & 1(f).

5. Robustness

The robustness of the assay method was with effect of buffer pH, pH adjusted ± 0.2 units (pH 2.8 and pH 3.2). The method complied with the system suitability parameters – System precision, No. Of Theoretical plates, Resolution and Tailing factor were with in the ICH specified limits.

6. System suitability parameters

System suitability parameters are fundamental criteria in Gas and Liquid Chromatography. These parameters are performed before each analysis to verify that the results obtained are accurate and precise.

The parameters are system precision, resolution, number of theoretical plates and Tailing factor. The data of these parameters are specified in Table No.1(g)

2. High Performance Thin Layer Chromatography

Izmailov and Shraiber (1938) first introduced the technique of thin layer chromatography for the separation of plant extracts using layer of alumina set on glass plate. TLC as a procedure for analytical adsorption chromatography was first introduced by Stahl (1958) who was mainly responsible for bringing out a standard equipment for preparing thin layers. Thin Layer chromatography can be used as a qualitative tool for separation of simple mixtures where low cost and simplicity are required, or else it can be used as a powerful separation tool for quantitative analysis, the later now referred to as HPTLC. It can simultaneously handle several samples even of divergent nature and composition.[24]

High performance Thin Layer Chromatography enables the most complicated separations. The HPTLC plates are prepared from optimized (e.g., particle size and particle size distribution) adsorbent layers and extremely even surfaces. These plates offer greater separation efficiency through smaller plate heights than the conventional TLC plates. Shorter analysis times, detection limits in the nanogram range with the UV absorption detection and in the picogram range with flourimetric detection are some additional advantages with these plates. [24]

Steps involved in HPTLC[25]

1. Selection of chromatographic layer
2. Sample and standard preparation

3. Layer pre-washing

4. Layer pre-conditioning

5. Application of sample and standard

6. Chromatographic development

7. Detection of spots

8. Scanning and Documentation of chromatic plate

Sample application.

The samples to be chromatographed are applied to the chromatogram layer. Volume precision and exact positioning are ensured by the use of a suitable instrument.

Chromatogram development

The solvent (mobile phase) migrates the predetermined distance in the layer (stationary phase) by capillary action. In this process, the samples are separated into fractions. After evaporation of the mobile phase, the fractions remain stored on the layer.

Chromatogram evaluation

The tracks (samples) are scanned in a densitometer with a light beam in the visible or ultraviolet range of the spectrum. Absorbance or fluorescence is

measured by diffuse reflectance. Alternatively to classical densitometry, the chromatogram can be evaluated by video technology. Additional operations such as per or post-chromatographic derivatisation can be performed as required.

HPTLC has become the accepted term for layer which

- are slightly thinner than conventional layers(0.20mm instead of 0.25 mm) and thus need less sample to show the same measuring result.

- have a smaller mean grain size – 7 instead of 12-20 μm and in particular a closer grain size distribution than conventional layers.

- hence give better resolution with a migration distance about 50% shorter-50mm as against 100-120mm.

- and have improved optical properties over conventional layers which gives better accuracy during densitometric evaluation.

A. INSTRUMENTS

High Performance Thin Layer Chromatograph

CAMAG HPTLC SCANNER II with wincats software

Linomat IV Sample applicator

Silicagel 60F 254 (MERK) precoated sheets

CAMAG Twin trough chamber

Balance: Denver Instruments

Centrifuge – Eltek Microspin TC 4815 D

Sonicator – Enertech Electronics

B. REAGENTS AND CHEMICALS

Toluene AR grade

Methanol AR grade

Chloroform AR grade

Triethylamine AR grade

Ethyl acetate AR

Formic acid AR

Ammonia AR

Silica gel G

Iodine

Active Pharmaceutical Ingredients – Finasteride and Tamsulosin Hydrochloride

Market sample of formulation from a local pharmacy

C. CHOICE OF SOLVENTS (Trials)

Initial trials were made with the prepared TLC plates, glass plates coated with Silicagel G. The spots were identified using iodine to confirm the resolution of the drugs from the mixture. Individual drug standards are also spotted to determine the Rf of each of the drugs.

Trial No.	Mobile Phase	Ratio	Observation
1.	Chloroform: EAC: Ammonia	7:2.5:0.5	The two drugs did not resolve much
2.	Chloroform: EAC: Ammonia	6:4:1.5	No proper resolution of the drugs from mixture spot.
3.	Chloroform: EAC: Water	6:3:1	No proper resolution of the drugs from mixture spot.
4.	Toluene: Chloroform: Water	7:2:1	Tailing of the spots
5.	Toluene: EAC: Methanol	6:3:1	The two drugs did not resolve much
6.	Toluene: Chloroform: Formic acid	6:3:1	No separation of spots
7.	Toluene: EAC: Formic acid	6:3:1	Separation of both drugs from the mixture. Distance travelled are: Solvent front – 7.25cm Finasteride – 2.4 cm; Rf: 0.331 Tamsulosin – 1.2 cm; Rf: 0.166

Trial No.	Mobile Phase	Ratio	Observation
8.	Toluene:Chloroform:Methanol	6:3:1	Both the drugs were resolved from the mixture spot. Distance travelled are: Solvent front – 8.5cm Finasteride – 3.8cm; Rf: 0.447 Tamsulosin – 2.4cm; Rf: 0.282
9.	Toluene:Chloroform:Methanol	6:2:2	Both the drugs were resolved from the mixture spot. Distance travelled are: Solvent front – 8.7cm Finasteride – 3.9cm; Rf: 0.448 Tamsulosin–2.55cm; Rf: 0.293
Trials with precoated sheets and scanning in HPTLC scanner			
10.	Toluene:Chloroform:Methanol	6:2:2	Streaking of the Tamsulosin spot. The peak shape was not proper.
11.	Toluene:Chloroform:Methanol: Triethylamine	6:2:2:1	Streaking of the Tamsulosin spot was eliminated. But the resolution of drugs from the mixture was reduced.
12.	Toluene:Chloroform:Methanol: Triethylamine	7:2:1:2	Streaking of the Tamsulosin spot was eliminated with good resolution of drugs

D. OPTIMISED CHROMATOGRAPHIC CONDITION

Stationary Phase: HPTLC Silicagel 60F 254 precoated (Merck)

Mobile Phase: Toluene: Chloroform: Methanol: Triethylamine (7:2:1:2.0)

Diluent: Methanol

Plate dimension: 20 x 10

Application mode: CAMAG Linomat IV

Development mode: Twin trough chamber

Saturation time: 20 minutes

Temperature: Ambient

Migration distance: 90mm

Distance between tracks: 10.0mm

Slit width: 5mm

Slit length: 5mm

Scanning speed: 4mm/sec

Source of radiation: Deuterium

Wavelength scanning: 230nm

Wavelength increment: 5nm

E. EXPERIMENTAL

Preparation of standard solution

About 125mg of Finasteride and 10mg of Tamsulosin Hydrochloride was weighed accurately and transferred into 25ml volumetric flask, dissolved and made up to the volume with methanol to get the stock solution. From this serial dilutions were made to obtain a final concentration ranging from 500 – 1500mcg/ml of Finasteride and 40-120mcg/ml of Tamsulosin Hydrochloride. Linearity was established in this concentration range and calibration curve was constructed, by injecting 25µl of the standard solutions. Linearity data are presented in Table No. 2(a), 2(b) and the graphs are presented as graph No. 2 (a), 2(b), 2(c) and 2(d)

Market Sample Analysis

The content of twenty capsules were accurately weighed and powdered. The powder equivalent to 10mg of Finasteride and 0.8mg of Tamsulosin Hydrochloride was accurately weighed and transferred into a 10ml volumetric flask. The content was dissolved in 8ml of methanol and subjected to sonication for 30 minutes. This solution was then allowed to stand for 10 minutes and made up to the volume with methanol. This final solution is centrifuged and exhibited the concentration of 1000mcg/ml of

Finasteride and 80mcg/ml of Tamsulosin Hydrochloride, which is used for further estimations by spotting 25µl.

The amount of drug present per capsule was calculated by adopting the following formula.

$$= \frac{\text{Area of Sample x Standard dilution x Average weight x Potency}}{\text{Area of Standard x Sample dilution x 100}}$$

The assay results are presented in the table No.2(c)

Recovery Studies

Recovery studies were carried out by addition of a known quantity of the standard drug to the pre-analyzed samples (50, 100 and 150% of target level) and the whole contents were reanalyzed by the proposed method.

Percentage recovery was calculated by using the following formula:

$$= \frac{\text{Amt. found in solution – Actual amt. of preanalysed sample}}{\text{Amount of standard added}} \text{ x } 100$$

The data are presented in table no. 2(e) and 2(f).

METHOD VALIDATION

1. Specificity

The Specificity is the ability to assess unequivocally the analyte in the presence of components that may be expected to be present, such as impurities, degradation products and matrix components. This definition implies the

identification tests, purity test and assays. The two drugs have specific retardation factor in the optimized chromatographic conditions.

2. Linearity and Range

The linearity and range of the analytical method is determined from the calibration curve and mathematical transformations proportional to the concentration of the analyte. The prepared standard stock solution is diluted to the concentration ranging from 50 to 150% (of 100% target level) of finasteride and Tamsulosin Hydrochloride. The data are presented in the table No. 2(a) and 2 (b).

3. Precision (Method Precision)

Precision was evaluated by quantifying homogenous sample in six times for intra-day assay precision equivalent to 1000µg/ml of Finasteride and 80µg/ml of Tamsulosin hydrochloride.

The amount of drug present per capsule was calculated by adopting the following formula.

$$= \frac{\text{Area of Sample x Standard dilution x Average weight x Potency}}{\text{Area of Standard x Sample dilution x 100}}$$

The assay results are presented in the table No.2(d)

4. Accuracy (Recovery)

To ensure the accuracy of the method, recovery studies were carried out by addition of a known quantity of the standard drug to the pre-analyzed samples (50, 100 and 150% of target level) and the whole contents were reanalyzed by the proposed method.

Percentage recovery was calculated by using the following formula:

$$= \frac{\text{Amt. found in solution} - \text{Actual amt. of preanalysed sample}}{\text{Amount of standard added}} \times 100$$

The data are presented in table no. 2(e) and 2(f).

5. Stability of drug solution

The standard solution and sample solution were spotted after 24 hours to study the stability of the analyte in solution. Finasteride and Tamsulosin Hydrochloride were stable for up to 24 hours.

6. System suitability parameters

System suitability parameters are fundamental criteria in Gas and Liquid Chromatography. These parameters (system precision, resolution, number of theoretical plates and Tailing factor) are performed before each analysis to verify that the results obtained are accurate and precise.

The parameters considered for HPTLC are system precision, resolution factor, retardation factor and Tailing factor. The data of these parameters are specified in Table No. 2(g)

3. UV-Visible Spectrophotometric method

(Q-Absorbance ratio method)

UV - VISIBLE SPECTROSCOPY involves the measurement of amount of ultra-violet radiation absorbed by a substance in the solution. The wavelength between 190 – 390 nm (practically 200 – 400 nm) is considered to be UV radiations/ region. Colored compounds absorb in visible range i.e. 400-800 nm.

The assay of an absorbing substance, can be carried out by using

a) Standard absorptivity value.

b) Use of calibration graph.

c) Single point standardisation.

The use of UV and visible spectroscopy for quantitative analysis employs the method of comparing the absorbance of standards and samples at a selected wavelength. The analysis of mixtures of two or more components is facilitated by activity of absorbance. Other applications include measurement of absorption of complexes to establish their composition. All chromogenic compounds are not suitable for quantitative measurements, i.e. the choice of the system and procedure depends largely on the chemistry of the species to be determined.[26]

Points to be considered in the selection of procedure include:

- Stability of absorbance with respect to time, variation of pH, ionic strength and temperature.

- Degree of selectivity of complexing agent includes the effect of other species likely to be present.

- Conformity to the Beer-Lambert's Law and plot calibration data for the range of concentration measured.

In multi-component formulations, the presence of two or more drugs in a formulation give rise to interference components which mutually interfere with each other in their estimation. For the simultaneous estimation of drugs in such formulations many techniques have been applied.

They are[27]

1) Simultaneous equation method
2) Absorbance ratio method
3) Derivative spectroscopy method.
4) Chemical Derivatisation Methods.
5) Multi-component mode of analysis.

ABSORBANCE RATIO OR Q-ABSORBANCE METHOD[27]

This method depends on the property of a substance, which obeys Beer's law at all wavelengths, the ratio of absorbance at any two wavelengths is a constant value independent of concentration or path length. Two different dilutions of the same sample give the same absorbance ratio; this ratio is referred to as a 'Q' value. In quantitative assay of two components in a admixture by the absorbance ratio method, absorbances are measured at two wavelengths: one being the λ_{max} of the components (λ_2) and the other being a wavelength of equal absorptivity of the two components (λ_1), i.e., iso-absorptive point. Two equations are constructed.

Equation 1: $A_1 = a_{x1} (C_x + C_Y)$

Equation 2: $C_x = Q_M\text{-}Q_Y / Q_x - Q_Y \times A_1 / a_{x1.}$

Accurate dilutions of the sample solution and standard solutions (of the individual drugs) are necessary for the accurate measurements of A_1 and a_{x1} respectively.

A_1 and A_2 are the absorbances of sample solution at λ_2 and λ_1

X and Y are the two components present in sample solution.

a_{x1} is the absorptivity of standard solution of component X at λ_1

A. INSTRUMENTS

UV-Visible Spectrophotometer – Shimadzu 1650 PC

Balance – Mettler AB 54

Sonicator – Enertech electronics

Centrifuge – Remi clinical Model 854/4

B. REAGENTS AND CHEMICALS

Alcohol AR grade

Distilled Water

Active Pharmaceutical Ingredients – Finasteride USP; Tamsulosin Hydrochloride

Market sample of formulation from a local pharmacy

C. EXPERIMENTAL

1. Devising the solvent for the drugs

Based on their solubility profile, the solvent mixture, ethanol 95% v/v and distilled water in ratio of 30:50 was selected for the spectrophotometric estimation.

2. Establishment of various parameters

(a) Determination of Absorption maximum – λ_{max} and isoabsorptive point

Preparation of Standard Solutions

About 25mg of Finasteride was accurately weighed and transferred to a 25ml volumetric flask, dissolved completely using ethanol: water (3:5) and further made it to volume with same solvent to get the stock solution. From this serial dilution was made to obtain a final concentration of 10µg/ml.

About 25mg of Tamsulosin hydrochloride was accurately weighed and transferred to a 25ml volumetric flask, dissolved completely using ethanol:water (3:5) and further made it to volume with same solvent to get the stock solution. From this serial dilution was made to obtain a final concentration of 10µg/ml.

The solutions were scanned separately in the wavelength range of 190 – 400nm and the spectrum was recorded for the individual standards. λ_{max} of Finasteride was found to 205nm and that of Tamsulosin Hydrochloride was 225nm. The overlay spectra of the two drugs show three isoabsorptive points**:** 217.7nm, 239nm and 260nm. Of these, 217.7 nm was chosen for the estimation.

(b) Beer's Law Plot and Linearity

From the stock solution of individual standard solutions of Finasteride and Tamsulosin Hydrochloride serial dilutions were made to obtain the concentrations ranging from 5-30µg/ml for Finasteride and 5-30µg/ml for Tamsulosin hydrochloride. The above solutions were scanned over the range of 190nm to 400nm and the resultant spectra were recorded. The response versus concentration obeyed linear relationship, the line of best fit was found out by using line of linear regression equation.

$Y = \alpha + \beta x$.

$$\alpha = \frac{(\Sigma Y)(\Sigma X^2) - (\Sigma X)(\Sigma XY)}{N\Sigma X^2 - (\Sigma X)^2}$$

$$\beta = \frac{N\Sigma XY - (\Sigma X)(\Sigma Y)}{N\Sigma X^2 - (\Sigma X)^2}$$

Where,

X	-	Concentration µg/ml
Y	-	Response corresponding to concentration X
α	-	Intercept
β	-	Slope

Residuals of the responses were calculated and plotted against the concentration (x axis). It was found that the points were scattered randomly around zero of x axis. Subsequently LOD and LOQ values were determined from the plot.

The data and graphs are presented as Table No, 3(a), 3(b) and Graph No.3(a), 3(b), 3(c) and 3(d) respectively.

LOD = 3.3 x Standard deviation of residuals / slope

LOQ = 10 x Standard deviation of residuals / slope

ABSORBANCE RATIO OR Q-ABSORBANCE RATIO METHOD

This method depends on the property of a substance, which obeys Beer's law at all wavelengths, the ratio of absorbance at any two wavelengths is a constant value independent of concentration or path length. In quantitative assay of two components in a admixture by the absorbance ratio method, absorbances are measured at two wavelengths: one being the λ_{max} of the components (λ_2) and the other being a wavelength of equal absorptivity of the two components (λ_1), i.e., iso-absorptive point. Two equations are constructed.

Equation 1: $A_1 = a_{x1} (C_x + C_Y)$

Equation 2: $C_x = Q_M\text{-}Q_Y / Q_x - Q_Y \times A_1 / a_{x1.}$

Market Sample analysis

Preparation of Sample solution

The content of twenty capsules were accurately weighed and powdered. The powder equivalent to 5mg of Finasteride and 0.4mg of Tamsulosin Hydrochloride was accurately weighed and transferred into a 50ml volumetric flask. The content was dissolved in 25ml of ethanol- water (3:5) solvent mixture and subjected to sonication for 20 minutes. To this solution 5ml of Tamsulosin Hydrochloride Standard solution (1mg/ml) was added, mixed well and then allowed to stand for 2 minutes and made up to the volume with the same solvent. This final solution is centrifuged for 5 minutes and had the concentration of 10mcg/ml of Finasteride and 10.8mcg/ml of Tamsulosin Hydrochloride, which is used for further estimations.

The amount of drug present per capsule was calculated by adopting the following formula.

$$= \frac{\text{Area of Sample x Standard dilution x Average weight x Potency}}{\text{Area of Standard x Sample dilution x 100}}$$

The assay results are presented in the table No.3(c)

Recovery Studies

Recovery studies were carried out by addition of a known quantity of the standard drug to the pre-analyzed samples (50, 100 and 150% of target level) and the whole contents were reanalyzed by the proposed method.

Percentage recovery was calculated by using the following formula:

$$= \frac{\text{Amt. found in solution} - \text{Actual amt. of preanalysed sample}}{\text{Amount of standard added}} \times 100$$

The data are presented in table no. 3(e) and 3(f)

METHOD VALIDATION

1. Specificity

The Specificity is the ability to assess unequivocally the analyte in the presence of components that may be expected to be present, such as impurities, degradation products and matrix components. This definition implies the identification tests, purity test and assays. The two drugs have specific $\lambda_{max.}$

2. Linearity and Range

The linearity and range of the analytical method is determined from the calibration curve and mathematical transformations proportional to the concentration of the analyte. The prepared standard stock solution is diluted to the concentration ranging from 50 to 150% (of 100% target level) of finasteride and Tamsulosin Hydrochloride. The data are presented in the Table No. 3(a) and 3 (b).

3. Precision (Method Precision, Intermediate Precision (Ruggedness))

Precision was evaluated by quantifying homogenous sample in six times for both intra-assay and inter-assay precision equivalent to 10µg/ml of Finasteride and 10.8µg/ml of Tamsulosin hydrochloride.

The amount of drug present per capsule was calculated by adopting the following formula.

$$= \frac{\text{Area of Sample x Standard dilution x Average weight x Potency}}{\text{Area of Standard x Sample dilution x 100}}$$

The assay results are presented in the Table No.3 (d)

4. Accuracy (Recovery)

To ensure the accuracy of the method, recovery studies were carried out by addition of a known quantity of the standard drug to the pre-analyzed samples (50, 100 and 150% of target level) and the whole contents were reanalyzed by the proposed method.

Percentage recovery was calculated by using the following formula:

$$= \frac{\text{Amt. found in solution – Actual amt. of preanalysed sample}}{\text{Amount of standard added}} \text{ x } 100$$

The data are presented in Table No. 3(e) and 3(f)

5. Stability of drug solution:

The standard solution and sample solution were scanned after 24 hours to study the stability of the analyte in solution. Finasteride and Tamsulosin Hydrochloride were stable for up to 24 hours.

RESULTS AND DISCUSSION

1. Reverse Phase High Performance Liquid Chromatography method

The analytical conditions for the HPLC method was established for simultaneous estimation of Finasteride and Tamsulosin hydrochloride in Pharmaceutical dosage forms. Various mobile phase systems were prepared and trials were made to determine appropriate chromatographic conditions.

Finasteride monograph is official in USP. In context to the method specified for the active pharmaceutical ingredient and formulation, this method for combination of Finasteride and Tamsulosin Hydrochloride was developed. As a result, the method focused on C18 column and mobile phase, Acetonitrile: buffer (pH adjusted to 3). Different ratios of this mobile phase, flow rates and UV detector wavlength in various combinations were tried out.

The trials carried out are as follows:

With ACN: Buffer (75:25 v/v) as mobile phase at 220nm and 1.0ml/min flow rate, a trial was carried out. The capacity factor of Tamsulosin Hydrochloride was 0.35. Finasteride peak was not significant comparatively.

Then the flow rate was changed as 0.75ml and UV wavelength to 230nm. Tamsulosin Hydrochloride achieved a retension time at 2.05 minutes.

A trial was carried out with ACN: Buffer (50:50 v/v) as mobile phase at 230nm and 0.75ml flow rate. The retension time of Tamsulosin and Finasteride

was 2.05 mins and 8.79 minutes respectively. When we applied this Retension time, the linearity was narrowed.

Further a trial was carried out with ACN: Buffer (60:40 v/v) as mobile phase at 230nm and 0.75ml flow rate. The retension time of Tamsulosin was 2.65 mins. The retension time of Finasteride was extended to 7.79 minutes.

In the expectation of better peak area and resolution, we tried out with ACN: Buffer (60:40 v/v) as mobile phase at 240nm and 0.75ml flow rate. But we were deprived of achieving the goal as the peak corresponding to Tamsulosin was not prominent.

At last, ACN: Buffer (60:40 v/v) as mobile phase, 230nm as UV detector wavelength and 0.8ml flow rate are finalized for the chromatographic method. The retention time achieved for Tamsulosin Hydrochloride was 2.65 minutes and for Finasteride was 7.15 minutes. Total runtime for an injection was achieved as 10 minutes. The trials were exhibited as graphs in the following pages.

The System precision achieved was % RSD = 0.1151 and 0.1051 for FINA and TAMS respectively< Limit: Not more than 2%>, Tailing factor for Finasteride was 1.35 and Tamsulosin Hydrochloride was 1.65 were within the limit <Limit: Not more than 2>, Resolution was 21.4 < Limit: Not less than 2.0> & No.of Theoretical plates were 4600 for Tamsulosin Hydrochloride and 12136 for Finasteride < Limit: Not less than 2500>

The Linearity of HPLC method developed for the assay was evaluated by injecting mixture of standard drugs, Finasteride and Tamsulosin Hydrochloride at a concentration ranging from 50 – 150mcg/ml of Finasteride and 4-12mcg/ml of Tamsulosin Hydrochloride. A linearity data indicating the slope, y-intercept values, correlation coefficient, LOD and LOQ are presented in table no. 1(a) and 1(b). The correlation coefficient was found to be 0.998 indicating the best linearity.

The precision for the Finasteride and Tamsulosin Hydrochloride was evaluated by using homogeneous sample in six times determinations for both intra day and inter day assay (100% target level). The data are presented as table No. 1(d).

The assay of market sample determined the content of Finasteride as 99.51% (of label claim – 5mg) with a confidence interval of ±0.1568 and the content of Tamsulosin Hydrochloride as 99.78% (of label claim – 0.4mg) with a confidence interval of ±0.1403 showing good precision.

The accuracy was determined, for Finasteride and Tamsulosin hydrochloride by fortifying sample and standard drug substance at a concentration ranging from 50% to 150% of target level. The data are presented as 1(e) and 1(f). Overall recovery determined the content of Finasteride as 99.69% (of label claim – 5mg) with a confidence interval of ±0.3124 and the content of Tamsulosin Hydrochloride as 99.2% (of label claim – 0.4mg) with a confidence interval of ±0.1797. This indicates that the method is capable of showing good accuracy and reproducibility.

2. High Performance Thin Layer Chromatography method

The analytical conditions for the HPTLC method was established for simultaneous estimation of Finasteride and Tamsulosin hydrochloride in Pharmaceutical dosage forms. Various mobile phase systems were prepared and trials were made to determine appropriate chromatographic conditions. Trials were carried out with the prepared TLC plates for determination of Rf of individual drugs.

The trials carried out for optimization of solvents are as follows.

Trials with the mobile phase of Chloroform: EAC: Ammonia in ratio of (7:2.5:0.5) and (6:4:1.5) were carried out. But the two drugs did not resolve much.

Trials with the mobile phase of Chloroform: EAC: water in ratio of (6:3:1) and (7:2:1) were carried out. But there was no proper resolution of two drugs from the mixture spot. The latter also showed tailing of the spot.

Trials were made with the mobile phase of Toluene: EAC: Methanol in ratio of (6:3:1) and trials were also carried out with Toluene: Chloroform: Formic acid (6:3:1). But there was no separation between spots.

Trials were then made with the mobile phase of Toluene: EAC: Formic acid in ratio of (6:3:1). Separation of both drugs was achieved. But the Rf of Finasteride was found to be 0.331 and that of Tamsulosin was 0.166.

Trials were then made with the mobile phase of Toluene: Chloroform: Methanol in ratio of (6:3:1). Separation of both drugs was achieved. But the Rf of Finasteride was found to be 0.447 and that of Tamsulosin was 0.282.

Trials were then made with the mobile phase of Toluene: Chloroform: Methanol in ratio of (6:2:2). Separation of both drugs was achieved. But the Rf of Finasteride was found to be 0.448 and that of Tamsulosin was 0.293.

Photo of the chromatogram with spots developed in Iodine chamber is exhibited as Photo No.1 in the following pages.

This combination of mobile phase was finalized for HPTLC and proceeded with precoated plates. A streaking was observed with Tamsulosin spot, which affected the peak shape.

Densitogram is presented as Photo No.2 in the following pages.

Then the ratio of the mobile phase was modified as Toluene:Chloroform: Methanol:Triethylamine (6:2:2:1). The scanning of the developed plates showed a good peak shape but had the resolution was not good.

Photo of the chromatoplate obtained with the trial is presented as Photo No.3 in the following pages along with the corresponding densitogram as Photo No.4

Then the ratio of the mobile phase was modified as Toluene:Chloroform: Methanol:Triethylamine (7:2:1:2). The scanning of the developed plates showed a good peak shape and good resolution as well.

Chromatoplate obtained with the trial (with better resolution of two drugs) is presented as Photo No.5 in the following pages along with the corresponding densitogram as Photo No.6

The tailing factor was found to be not more than 2.0 and the Retardation factor was more than 0.2. Resolution factor was more than 2.0. The values are presented in the Table No.2(g) in the following pages.

The Linearity of HPTLC method developed for the assay was evaluated by injecting mixture of standard drugs, Finasteride and Tamsulosin Hydrochloride at a concentration ranging from 500 – 1500mcg/ml of Finasteride and 40-120mcg/ml of Tamsulosin Hydrochloride. A summary of the data indicating the slope, y-intercept values and correlation coefficient are presented in table no. 2(a) and 2(b). The correlation coefficient was found to be greater than 0.99.

The precision for the Finasteride and Tamsulosin Hydrochloride was evaluated by using homogeneous sample in six times determinations for intraday assay (100% target level). The data are presented as table No. 2(c).

The assay of market sample determined the content of Finasteride as 99.68% (of label claim – 5mg) with a confidence interval of ±0.0217 and the

content of Tamsulosin Hydrochloride as 99.85% (of label claim – 0.4mg) with a confidence interval of ± 0.2791.

The accuracy was determined, for Finasteride and Tamsulosin hydrochloride by fortifying sample and standard drug substance at a concentration ranging from 50% to 150% of target level. The data are presented as 2(e) and 2(f). Overall recovery determined the content of Finasteride as 99.48% (of label claim – 5mg) with a confidence interval of ±0.2269 and the content of Tamsulosin Hydrochloride as 99.57% (of label claim – 0.4mg) with a confidence interval of ±0.3021.

3. UV-Visible Spectrophotometric method (Q absorbance ratio)

The analytical conditions for the UV Spectroscopy by Q-absorbance method was established for simultaneous estimation of Finasteride and Tamsulosin Hydrochloride.

Based on the solubility profile of Finasteride and solubility profile of Tamsulosin Hydrochloride, the solvent was devised. The solvent used was mixture of ethanol 95%v/v and water in the ratio (3:5). Other ratios were not suitable as the drugs had different solubility profiles.

λ_{max} of Finasteride was determined as 205nm and λ_{max} of Tamsulosin Hydrochloride was determined as 225nm (presented as Spectrum No. 3(a) and 3(b) respectively in the following pages. Both the drugs obeyed Beers' Law within the concentration range of 5-30mcg. The Calibration curve and overlay spectra for concentrations of the drug within Beer's Law range are exhibited in the following pages as Graph No. 3(a), 3(b), 3(c) and 3(c) and Spectrum No. 3(a) and 3(b) respectively.

The overlay spectra of Finasteride and Tamsulosin Hydrochloride with the same concentration, determined three isoabsorptive points, which are 217.7nm, 239nm and 260nm. Of these 217.7nm (as λ_1) was suitable for Q-absorbance method with

λ_{max} of Tamsulosin Hydrochloride (as λ_2).

Two equations,

Equation 1: $A_1 = a_{x1} (C_x + C_Y)$;

Equation 2: $C_x = Q_M\text{-}Q_Y / Q_x - Q_Y \times A_1 / a_{x1}]$,

were devised based on which the concentration of Finasteride and Tamsulosin Hydrochloride in market sample were determined.

The regression equation was constructed with the slope and intercept values obtained from the linearity data. The correlation coefficient of Standard drugs were greater than 0.99. LOD, LOQ and Sandell's sensitivity were determined from the linearity and calibration data and presented as Table No. 3(a) and 3(b).

The assay of Finasteride was found to be 100.44% and that of Tamsulosin Hydrochloride was found to be 100.35% indicating good precision and repeatability.

The recovery studies also show that there is a reliable accuracy indicating the %recovery as 100.339% and 99.397% with the confidence interval (95%) as ±0.4550 and ±0.32099 for Finasteride and Tamsulosin Hydrochloride respectively showing good accuracy.

SUMMARY AND CONCLUSION

Analysts are forever in search of rapid, sensitive and accurate methods of analysis, that are viable in routine quantitative study.

This project was focused in the method development for the simultaneous quantification of the pharmaceutical dosage form of Finasteride and Tamsulosin Hydrochloride. It was successful in achieving three different analytical techniques, namely, 1) High Performance Liquid Chromatography (HPLC), 2) High Performance Thin Layer Chromatography (HPTLC) and 3) Q-absorbance ratio method by UV Spectroscopy for the routine analysis for the combination dosage forms.

The HPLC method, proved to have high selectivity, precision and accuracy. The drugs were eluted by C18, ODS column (Phenomenex), using Acetonitrile: Buffer (pH adjusted to 3.0 ± 0.2 with Orthophosphoric acid) in the ratio of 60:40 as mobile phase and UV detector at wavelength of 230nm. The retension time of Finasteride was 7.11 minutes and that of Tamsulosin Hydrochloride was 2.65 minutes.

The HPTLC method developed for the formulation proved to be easiest and quick method of analysis. The method was developed using CAMAG HPTLC scanner and Linomat applicator, with a mobile phase combination of Toluene: Chloroform: Methanol: Triethylamine in the ratio of 7:2:1:2 at 230nm as scanning wavelength. The retardation factor of Tamsulosin Hydrochloride was 0.35 and that of Finasteride was 0.78.

Q-absorbance ratio method developed for the dosage form, proved to be easy and economical method for the rapid evaluation of drug components utilizing λmax of Tamsulosin Hydrochloride and Isoabsorptive point of both the drugs.

The linearity data was subjected to linear regression analysis in order to confirm the linear relationship between concentration and response.

All the three analytical techniques developed were subjected to method validation as specified in ICH guidelines. The analytical performance characteristics were established and the values were found to be within the specified limits as recommended.

Quantitative estimation was subjected to statistical analysis. %RSD value obtained was below 2 indicates precision of the method. The low standard error value indicates accuracy of the method.

Thus the methods developed were found to be simple, specific, precise, linear, accurate and were reliable & reproducible for simultaneous quantification of Finasteride and Tamsulosin Hydrochloride in pharmaceutical dosage forms.

BIBLIOGRAPHY

1. **Principles of Instrumental analysis**: *Authors,* ***Douglas A. Skoog, F. James Holler, and Stanley R. Crouch,*** 5th edition, Thomson Brooks/Cole; 1997; pages. 674 - 675

2. ***The United States pharmacopoeia 29***, United States pharmacopoieal convention Inc., Rock Ville, 2005; pages.3050-3052.

3. ***The United States pharmacopoeia 29***, United States pharmacopoieal convention Inc., Rock Ville, 2005; pages.2647-2649.

4. ***Indian Pharmacopoeia***, published by controller of publications, New Delhi, Vol-II, 1996; pages A65 –A68.

5. ***Merck corp Inc, USA.,*** published *Patient Information Leaflet, Propecia tablets 1mg* in NDA 20-788/S-002, S-010 & S-011; pages 3-16.

6. ***Astellas Pharma Inc., Tokyo,*** published a revised edition of *Patient Information Leaflet, Flomax capsules 0.4mg* dated July 19, 2006**.**

7. ***Carlin-JR, Christofalo-P, Vandenhenvel-WJ;*** High performance liquid chromatography determination of N-(2-methyl-2 -propyl)-3-oxo-4-aza-5alpha-androst-1-ene-17beta-carboxamide, a 4-azasteroid, in human plasma from a Phase I study, Journal of Chromatography, Journal of Chromatography; 1988; 427(May 13); pages 79-91.

8. ***Soeishi-Y, Kobori-M, Kobayashi-SI, Higuchi-S;*** Sensitive method for the determination of Tamsulosin in human plasma using high performance liquid chromatography with fluorescence detection; Journal of Chromatography and Biomedical Applications; 1990; 533(Nov 30); pages 291-296.

9. ***Constanzer-ML,Matuszewski-BK, Bayne-WF;*** High performance liquid chromatographic method for the determination of finasteride in human plasma at therapeutic doses; Journal of Chromatography and Biomedical Applications; 1991; 566(May3); pages 127-134.

10. ***Ryan-JA, Compton-SV, Brooks-MA, Compton-;*** Rapid verification of identity and content of drug formulations using mid-infrared spectroscopy; Journal of Pharmaceutical and Biomedical Analysis; 1991; 9(4); pages 303-310.

11. ***Hiroshi Matsushima, Ken-ichi Takanuki, Hidetaka Kamimura, Takashi Watanabe and Saburo Higuchiry***; Highly sensitive method for the determination of tamsulosin hydrochloride in human plasma dialysate, plasma and urine by high-performance liquid chromatography–electrospray tandem mass spectrometry; Journal of Chromatography-B Biomedical Sciences and Applications; 1997; 695 (Aug 1st) (2); pages 317-327.

12. ***Syed-AA, Amshumalip-MK;*** LC determination of finasteride and its application to storage stability studies; Journal of Pharmaceutical and Biomedical Analysis; 2001; 25(5-6); pages 1015-1019.

13. ***Ilango-K, Valentina-P, Lakshmi-KS;*** Spectrophotometric method for the estimation of finasteride in tablets; Indian Journal of Pharmaceutical Sciences; 2002; 64(2); pages 174-175.

14. ***Loi-AL, Rueff-DM, Sappenfield-SM, Burke-JD;*** A retrospective chart review to facilitate the conversion of tamsulosin to formulary alpha-blockers in the treatment of benign prostatic hypertrophy; ASHP-Midyear-Clinical-Meeting; 2002; 37(Dec); P-167R

15. ***Li-XY, Ding-L, Li-LM, Hao-XY, Zhang-ZX;*** Determination of finasteride in human plasma by HPLC-MS; Acta- Pharmaceutica-Sinica-Yao-Hsuch-Pao; 2003; 38(6); NIL_0005

16. ***Zhang-ZF, Ding-L; Li-LM; Hao-XY; Zhang-ZX;*** The chiral separation of tamsulosin isomers by high performance liquid chromatography using cellulose tris (3,5-dimethylphenylcarbamate) as a chiral stationary phase; Journal of Pharmaceutical and Biomedical Analysis; 2004; 34(3); pages 689-693.

17. ***Maier -V, Horakova-J, Petr-J, Tesarova-E, Sevcik-J;*** Chiral separation of tamsulosin by capillary electrophoresis; Journal of Pharmaceutical and Biomedical Analysis; 2005; 39(3-4); pages 691-696.

18. ***Hulya Demir, Aysen Cucu, Serap Sakarya;*** Determination of finasteride in the tablet form by liquid chromatography and its analytical method validation; Analytica Chimica Acta; 2006; 557 (Jan31st) (1-2); pages 252-255.

19. ***K. Basavaiah B.C., Somashekar , U. R. Anilkumar , V. Ramakrishna;*** Sensitive bromatometric assay methods for finasteride in pharmaceuticals; Ecletica Quimica (Instituto de Quimica/ UNESP Caix)*;* 2006.

20. ***Pekka Keski-Rahkonen, Olavi Pärssinen, Esa Leppänen, Timo Mauriala, Marko Lehtonen and Seppo Auriola;*** Determination of tamsulosin in human aqueous humor and serum by liquid chromatography–electrospray ionization tandem mass spectrometry; Journal of Pharmaceutical and Biomedical Analysis; 2007; 43 (17th Jan) (2); pages 606-612.

21. ***The United States pharmacopoeia 29;***Official Monograph of Finasteride (API and Formulations); 2005; pages 907-909.

22. ***Principles of Instrumental analysis***; Authors: ***Douglas A. Skoog, F. James Holler, and Stanley R. Crouch,*** 5th edition, Thomson Brooks/Cole, 1997; page no.726

23. ***Practical Pharmaceutical Chemistry;*** Authors: ***Beckett A.H., and Stenlake J.B.,*** 4th edition, CBS publishers and distributors, 1997, Vol II; page no. 157.

24. *Instrumental methods of Chemical analysis;* Authors: ***Gurdeep R. Chatwal, Sham K. Anand,*** 5th Edition, Himalaya Publishing House, 2005; pages 2.599 - 2.615.

25. ***Dr. S. N. Meyyanathan***, Basic Prinicples of HPTLC, Pharmainfo.net; page 3.

26. *Hanssen*., *Pharmaceutical analysis;* ***Takeru Higuchi, and Einar Brochmann***, CBS Publishers and Distributors, New Delhi. 1997; page no.15.

27. ***Practical Pharmaceutical Chemistry;*** Authors: ***Beckett A.H., and Stenlake J.B.,*** 4th edition, CBS publishers and distributors, 1997, Vol II; pages 275 – 300.

28. ***K. Raja Rajeswari et al;*** RP-HPLC method for the simultaneous determination of Atorvastatin and Amlodipine in Tablet dosage form; Indian Journal of Pharmaceutical Sciences; 2006; (March –April); pages 275-277.

29. ***M. Gandhimathi, T.K. Ravi et al*****;** HPTLC method for the Simultaneous estimation of Tizanidine and Rofecoxib in Tablets; Indian Journal of Pharmaceutical Sciences; 2006; (March-April); pages 234-236.

30. ***Devarajan and Lakshmi Siva subramanian*****;** Simultneous spectrophotometric determination of Valdecoxib and Tizanidine in Tablets; Indian Journal of Pharmaceutical Sciences; 2006; (March – April); pages 240-242.

31. Meena Tiwari et al; Simultaneous spectrometric estimation of Valdecoxib and Paracetamol in Tablet formulations; Indian Journal of Pharmaceutical Sciences; 2006; (May-June); pages 370-373.

32. ***Deepti Jain and M.R. Khan*****;** Simultaneous Spectrophotometric determination of Atorvastatin Calcium and Amlodipine Besylate in tablets; Indian Journal of Pharmaceutical Sciences; 2006; (July-August); pages 546-548.

33. ***Deepti Jain et al*****;** Simultaneous Spectrophotometric estimation of Famotidine and Domperidone in Combined Tablet dosage form; Indian Journal of Pharmaceutical Sciences; 2006; (July-August); pages 503-505.

34. ***C.S. Ramaa et al***; Reverse Phase-High Performance Liquid Chromatographic determination of Tizanidine and Valdecoxib in Tablets; Indian Journal of Pharmaceutical Sciences; 2006; (July-August); pages 514-516.

35. ***S.L.Bodhankar and K.M. Patil;*** Validated HPTLC method for Simultaneous estimation of Phenytoin Sodium, Phenobarbitone Sodium and Carbamazepine in Tablet Dosage forms; Indian Journal of Pharmaceutical Sciences; 2005; (May-June); pages 351-355.

36. ***N. Udupa et al***; Simultaneous RP-HPLC estimation of Paracetamol and Rofecoxib in Tablets; Indian Journal of Pharmaceutical Sciences; 2005; (March-April); pages 247-249.

37. ***S.G. Wadodkar et al;*** Simultaneous High Performance Thin Layer Chromatographic estimation of Lamivudine and Stavudine in Tablet Dosage forms; Indian Journal of Pharmaceutical Sciences; 2005; (January-February); pages 96-97.

38. ***A.R. Bhat et al;*** RP-HPLC determination of Zidovudine and Lamivudine in Tablet dosage form; Indian Journal of Pharmaceutical Sciences; 2005; (January-February); pages 110-112.

39. ***Trivedi et al;*** Simultaneous Spectrophotometric methods for the estimation of Nimesulide and Tizanidine in a Tablet dosage form; Indian Journal of Pharmaceutical Sciences; 2005; (July-August); pages 501-504.

40. ***P.N. Pai et al;*** HPLC method for Simultaneous estimation of Rofecoxib and Tizanidine hydrochloride in Tablets; Indian Journal of Pharmaceutical Sciences; 2005; (July-August); pages 504-505.

41. ***Mishra P, Dolly Archana*****;** Simultaneous determination of Clopidogrel and Aspirin in Pharmaceutical dosage forms; Indian Journal of Pharmaceutical Sciences; 2006; (3); pages 365-368.

Photo No.1 Photo of the chromatogram with spots developed in Iodine chamber

Photo No.2: Densitogram with improper peak shape

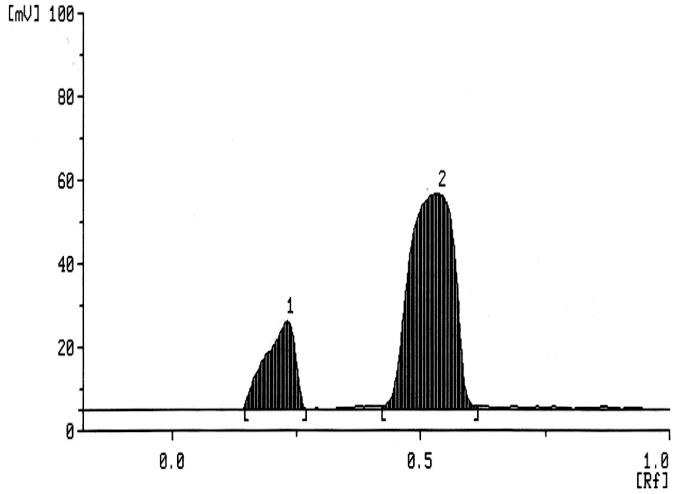


Photo No.3: Photo of the Chromato-plate with minimum resolution between two drugs (FINA and TAMS)

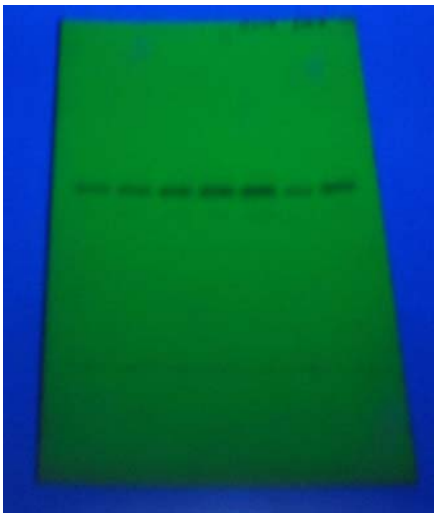


Photo No.4: Densitogram corresponding to the Chromato plate in Photo No.3

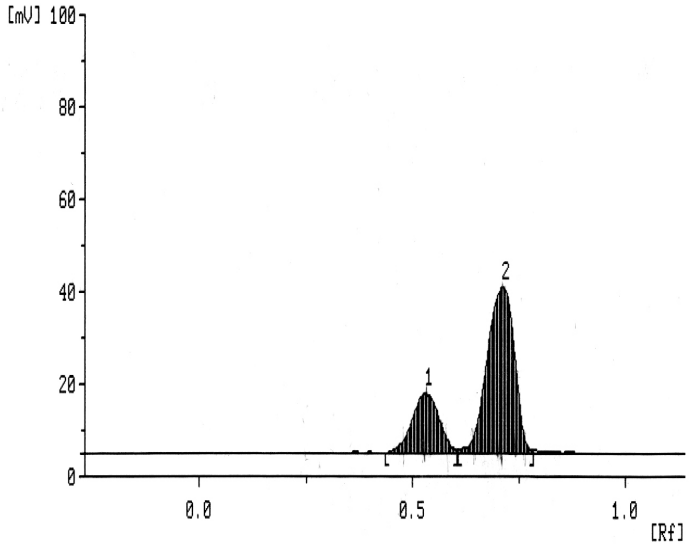


Photo No.5: Photo of the chromato- plate with good resolution of 2 distinct spots of the two drugs

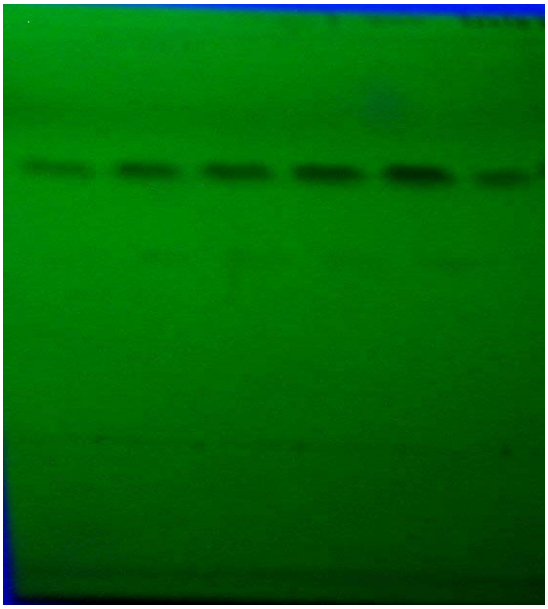


Photo No.6: Densitogram corresponding to the Chromato plate in Photo No.5

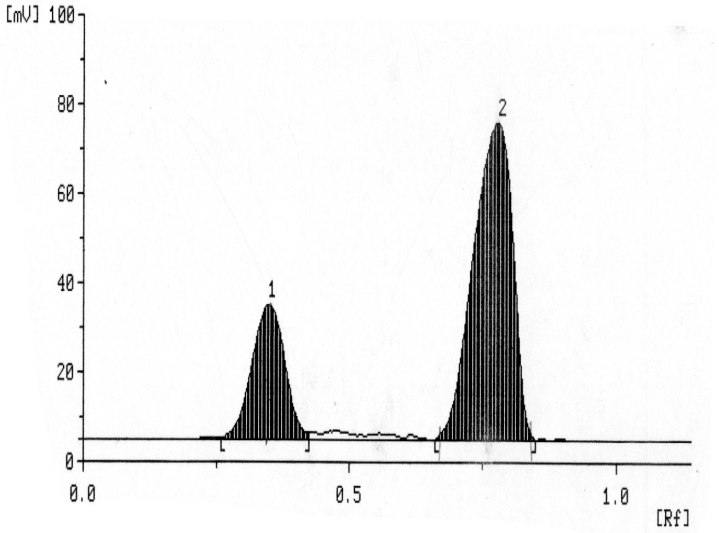


TABLE NO. 1(a): LINEARITY DATA OF FINASTERIDE (FINA) AND TAMSULOSIN HYDROCHLORIDE (TAMS) BY HPLC

S.No	Finasteride		Tamsulosin Hydrochloride	
	Concentration (mcg/ml)	Peak Area	Concentration (mcg/ml)	Peak Area
1.	50	878.599	4	233.451
2.	75	1442.121	6	350.144
3.	100	1801.245	8	466.283
4.	125	2401.502	10	575.461
5.	150	2715.651	12	687.236

TABLE NO. 1(b): ANALYTICAL PERFORMANCE PARAMETERS OF FINA AND TAMS FOR HPLC

Parameters	Finasteride	Tamsulosin Hydrochloride
Slope	18.534	57.313
y-Intercept	-1.989	3.343
Correlation coefficient	0.9981	0.9998
Limit of Detection (µg/ml)	10.962	0.193
Limit of Quantification (µg/ml)	33.218	0.584

TABLE NO. 1(c): ANALYSIS OF PHARMACEUTICAL FORMULATIONS BY HPLC

Label Claim: Finasteride 5mg and Tamsulosin Hydrochloride 0.4mg per capsule

Sample No.	FINA				TAMS			
	Amount esimated in mg/capsule*	%RSD	SE	CI	Amount estimated in mg/capsule*	%RSD	SE	(95%) CI
1.	5.0014	0.5858	0.01686	±0.0331	0.4012	0.5228	0.00121	±0.0024
2.	4.975				0.4006			
3	4.960				0.3973			

*Each value is an average of six determinations.

TABLE NO. 1(d):
PRECISION DATA (Repeatability and Intermediate Precision)

Sample No.	FINA (Amount estimated in mg/capsule)		TAMS (Amount estimated in mg/capsule)	
	INTRA DAY	INTER DAY	INTRA DAY	INTER DAY
1.	4.962	5.001	0.3968	0.4012
2.	4.968	4.997	0.3964	0.4005
3.	4.9767	4.973	0.3978	0.3978
4.	4.9975	4.9735	0.4012	0.4018
5.	4.944	4.960	0.4006	0.3973
6.	4.9805	4.9725	0.3954	0.4025
Mean: 4.9754			**Mean:** 0.3991	
%RSD: 0.3381			**%RSD:** 0.0024	
SE: 0.005			**SE:** 0.010	
(95%)CI: ±0.01			**(95%) CI:** ±0.0140	

TABLE NO. 1(e): RECOVERY STUDIES (Accuracy data)

1	Conc. levels	FINA			TAMS		
		Amt of Std added (mg)	Amt of Std. Recovered (mg)	% Recovery	Amt of Std added (mg)	Amt of Std. Recovered (mg)	% Recovery
1.	50%	12.53	12.59	100.48	1.010	1.0001	99.02
2.		12.53	12.563	100.26	1.010	1.0021	99.22
3.		12.53	12.535	100.28	1.010	1.0087	99.87
4.	100%	37.59	37.36	99.39	3.030	3.0187	99.63
5.		37.59	37.305	99.24	3.030	3.0027	99.10
6.		37.59	37.415	99.53	3.030	3.0079	99.27
7.	150%	62.65	62.125	99.36	5.050	5.0052	99.10
8.		62.65	62.208	99.29	5.050	5.013	99.28
9.		62.65	62.268	99.39	5.050	5.011	99.23

TABLE NO. 1(f): EVALUATION OF ACCURACY DATA

S.No.	Concentration Levels	% Recovery of FINA	% Recovery of TAMS
1.	50%	100.48	99.02
2.		100.26	99.22
3.		100.28	99.87
4.	100%	99.39	99.63
5.		99.24	99.10
6.		99.53	99.27
7.	150%	99.36	99.10
8.		99.29	99.28
9.		99.39	99.23
Mean		99.69	99.30
%RSD		0.4798	0.2768
SE		0.1594	0.0917
(95%) CI		±0.3124	±0.1797

TABLE NO. 1(g): SYSTEM SUITABILITY DATA

Parameters	Values		Acceptance Criteria
	FINA	TAMS	
% RSD	0.1151	0.1015	Not more than 2.0%
Theoretical plates	12150	4600	Not less than 2500
Tailing factor	1.35	1.65	Not more than 2.0
Resolution	21.4		Not less than 2.0

TABLE NO. 2(a): LINEARITY DATA OF FINA AND TAMS BY HPTLC

S.No	Finasteride		Tamsulosin Hydrochloride	
	Concentration (mcg/spot)	Peak Area	Concentration (mcg/spot)	Peak Area
1.	12.5	1226.3	1.0	419.0
2.	18.75	1645.7	1.5	635.3
3.	25.0	2250.4	2.0	853.2
4.	31.25	2780.6	2.5	1042.7
5.	37.5	3340.1	3.0	1261.1

TABLE NO. 2(b): ANALYTICAL PERFORMANCE PARAMETERS OF FINA AND TAMS FOR HPTLC

Parameters	Finasteride	Tamsulosin Hydrochloride
Slope	88.168	419.926
y-Intercept	37.007	2.007
Correlation coefficient	0.9982	0.9996
Limit of Detection (µg/spot)	1.770	0.054
Limit of Quantification (µg/spot)	5.363	0.164

TABLE NO. 2(c): ANALYSIS OF PHARMACEUTICAL FORMULATIONS BY HPTLC

Label Claim: Finasteride 5mg and Tamsulosin Hydrochloride 0.4mg per capsule

Sample No.	FINA				TAMS			
	Amount esimated in mg/capsule*	%RSD	SE	CI	Amount estimated in mg/capsule*	%RSD	SE	(95%) CI
1.	4.9835	0.1758	0.0051	±0.01	0.4010	0.4793	0.0011	±0.0022
2.	4.974				0.4001			
3	4.9915				0.3971			

*Each value is an average of six determinations.

TABLE NO. 2(d): PRECISION DATA (Intra day Assay)

Sample No.	FINA (Amount estimated in mg/capsule)	TAMS (Amount estimated in mg/capsule)
1.	4.9695	0.3976
2.	5.001	0.3978
3.	5.024	0.4010
4.	4.984	0.3987
5.	4.968	0.3973
6.	4.957	0.3992
Mean: 4.9839		**Mean:** 0.3986
%RSD: 0.4984		**%RSD:** 0.3500
SE: 0.011		**SE:** 0.1424
(95%) CI: ±0.02174		**(95%) CI:** ±0.2791

TABLE NO. 2(e): RECOVERY STUDIES (Accuracy data)

Sample No.	Conc. levels	FINA			TAMS		
		Amt of Std added (mg)	Amt of Std. Recovered (mg)	% Recovery	Amt of Std added (mg)	Amt of Std. Recovered (mg)	% Recovery
1.	50%	12.50	12.41	99.26	1.000	1.003	100.35
2.		12.50	12.395	99.16	1.000	0.995	99.50
3.		12.50	12.48	99.88	1.000	0.9889	98.89
4.	100%	37.50	37.33	99.55	3.000	2.9804	99.35
5.		37.50	37.155	99.10	3.000	2.997	99.9
6.		37.50	37.453	99.87	3.000	2.972	99.10
7.	150%	62.50	61.90	99.05	5.000	5.015	100.3
8.		62.50	62.23	99.57	5.000	4.980	99.6
9.		62.50	62.43	99.88	5.000	4.957	99.14

TABLE NO. 2(f): EVALUATION OF ACCURACY DATA

S.No.	Concentration Levels	% Recovery of FINA	% Recovery of TAMS
1.	50%	99.26	100.35
2.		99.16	99.50
3.		99.88	98.89
4.	100%	99.55	99.35
5.		99.10	99.9
6.		99.87	99.10
7.	150%	99.05	100.3
8.		99.57	99.6
9.		99.88	99.14
Mean		99.48	99.57
%RSD		0.3492	0.4644
SE		0.1158	0.1541
(95%) CI		±0.2269	±0.3021

TABLE NO. 2(g): SYSTEM SUITABILITY DATA

Parameters	Values		Acceptance Criteria
	FINA	TAMS	
% RSD	0.4984	0.5014	Not more than 2.0%
Rf	0.35	0.78	Not less than 0.2
Tailing factor	0.985	0.92	Not more than 2.0
Resolution	2.56		Not less than 2.0

TABLE NO. 3(a): LINEARITY DATA OF FINA AND TAMS BY UV SPECTROPHOTOMETRY

S.No	Finasteride		Tamsulosin Hydrochloride	
	Concentration (mg/ml)	Absorbance at λmax (205 nm)	Concentration (mcg/ml)	Absorbance at λmax (225 nm)
1.	5	0.212	5	0.240
2.	10	0.389	10	0.372
3.	15	0.603	15	0.591
4.	20	0.751	20	0.748
5.	25	0.972	25	0.942
6.	30	1.111	30	1.094

TABLE NO. 3(b): ANALYTICAL PERFORMANCE PARAMETERS OF FINA AND TAMS FOR UV SPECTROPHOTOMETRY

Parameters	Finasteride	Tamsulosin Hydrochloride
Slope	0.0373	0.0362
y-Intercept	0.0181	0.0272
Correlation coefficient	0.9988	0.9985
Limit of Detection (µg/ml)	1.770	2.024
Limit of Quantification (µg/ml)	5.363	6.133
Sandell's Sensitivity ($mcg/cm^2/0.001$)	0.02559	0.02563

TABLE NO. 3(c): ANALYSIS OF PHARMACEUTICAL FORMULATIONS BY UV SPECTROPHOTOMETRY

Label Claim: Finasteride 5mg and Tamsulosin Hydrochloride 0.4mg per capsule

Sample No.	FINA				TAMS			
	Amount esimated in mg/capsule*	%RSD	SE	(95%) CI	Amount estimated in mg/capsule*	%RSD	SE	(95%) CI
1.	5.024	0.8185	0.0238	±0.0466	0.4012	0.7108	0.0017	±0.0032
2.	5.005				0.4053			
3	5.084				0.3998			

*Each value is an average of six determinations.

TABLE NO. 3(d): PRECISION DATA (Repeatability and Intermediate Precision)

Sample No.	FINA (Amount estimated in mg/capsule)		TAMS (Amount estimated in mg/capsule)	
	INTRA DAY	INTER DAY	INTRA DAY	INTER DAY
1.	5.024	5.001	0.4009	0.4012
2.	5.005	5.018	0.4050	0.4005
3.	5.011	5.098	0.3998	0.3987
4.	5.076	5.0167	0.4015	0.4018
5.	5.047	4.9899	0.4046	0.3968
6.	4.989	4.9725	0.4025	0.4031
Mean: 5.0207			**Mean:** 0.4014	
%RSD: 0.7297			**%RSD:** 0.5798	
SE: 0.0106			**SE:** 0.00067	
(95%) CI: ±0.02073			**(95%) CI:** ±0.00132	

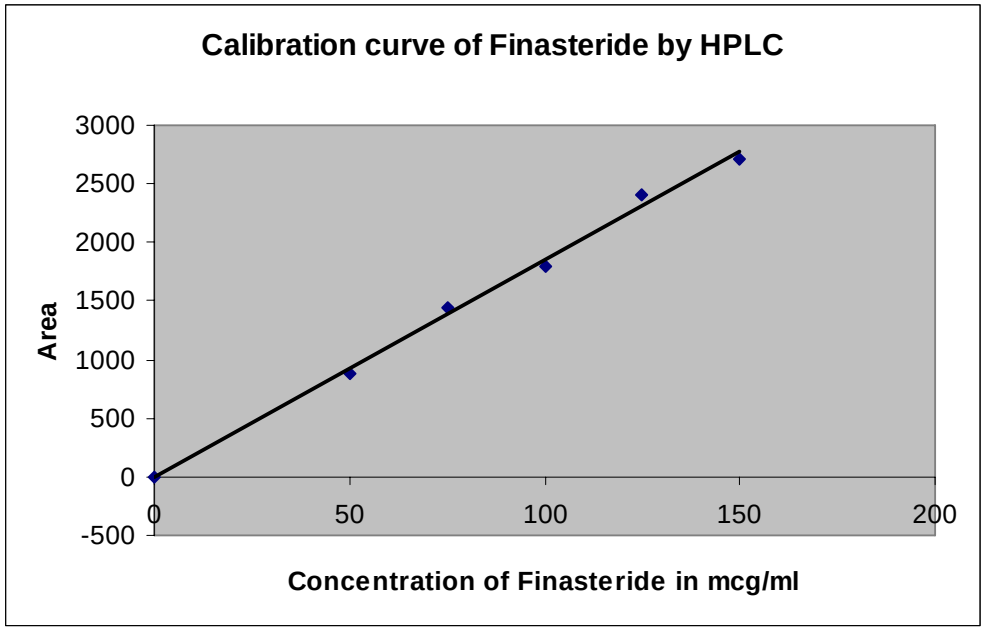
TABLE NO. 3(e): RECOVERY STUDIES (Accuracy data)

Sample No.	Conc. levels	FINA			TAMS		
		Amt of Std added (mg)	Amt of Std. Recovered (mg)	% Recovery	Amt of Std added (mg)	Amt of Std. Recovered (mg)	% Recovery
1.	50%	2.5	2.525	101.00	2.5	2.491	99.64
2.		2.5	2.541	101.64	2.5	2.476	99.22
3.		2.5	2.498	99.92	2.5	2.457	99.04
4.	100%	7.5	7.514	100.19	7.5	7.417	98.89
5.		7.5	7.478	99.71	7.5	7.509	100.12
6.		7.5	7.551	100.68	7.5	7.428	99.04
7.	150%	12.5	12.43	99.44	12.5	12.407	99.26
8.		12.5	12.57	100.56	12.5	12.53	100.24
9.		12.5	12.489	99.91	12.5	12.39	99.12

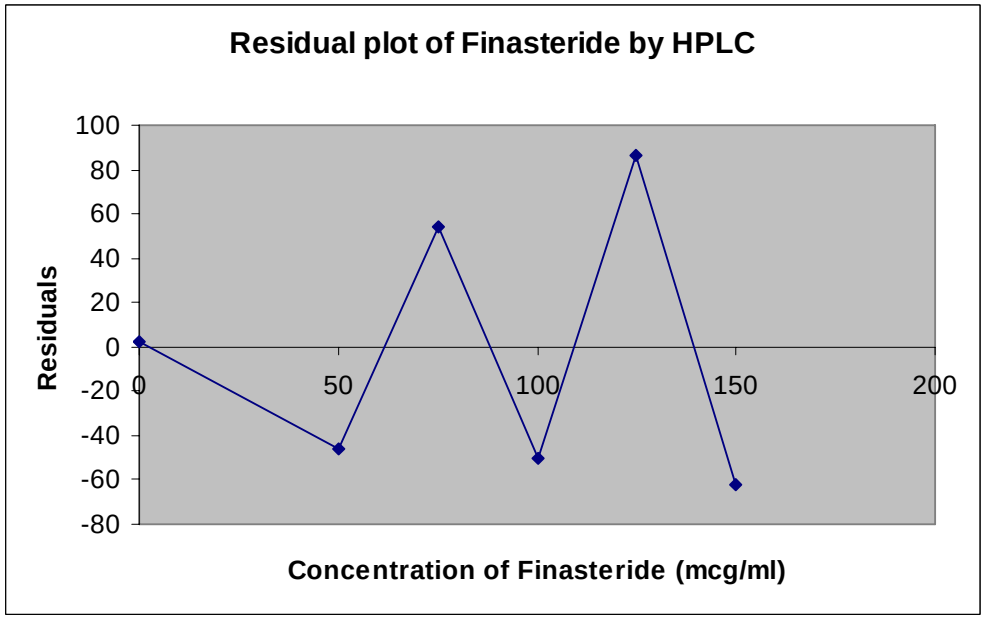
TABLE NO. 3(f): EVALUATION OF ACCURACY DATA

S.No.	Concentration Levels	% Recovery of FINA	% Recovery of TAMS
1.	50%	101.00	99.64
2.		101.64	99.22
3.		99.92	99.04
4.	100%	100.19	98.89
5.		99.71	100.12
6.		100.68	99.04
7.	150%	99.44	99.26
8.		100.56	100.24
9.		99.91	99.12
Mean		100.339	99.397
%RSD		0.6941	0.4943
SE		0.2321	0.1638
(95%) CI		±0.4550	±0.32099

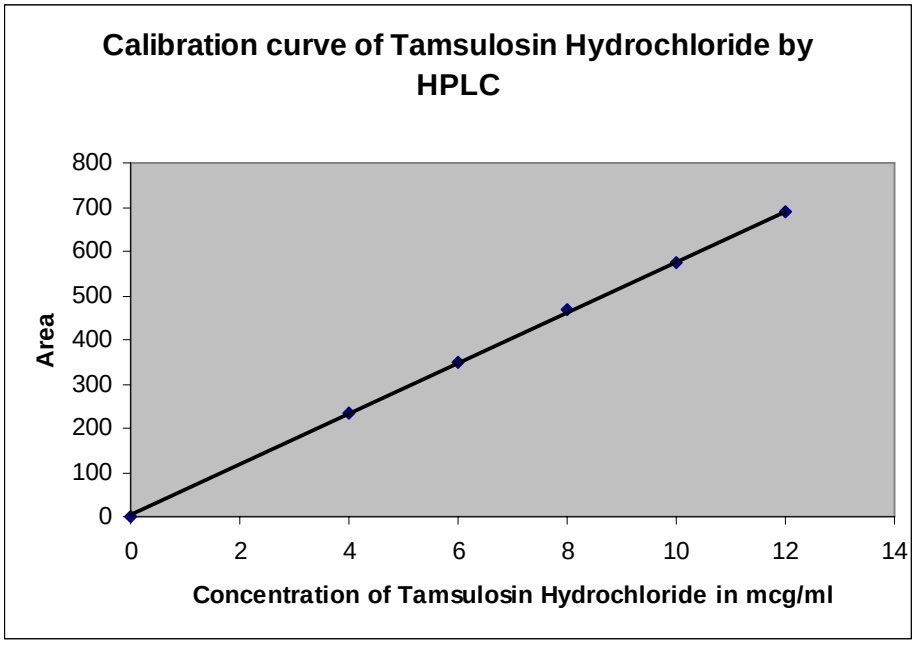
Graph No. 1(a)



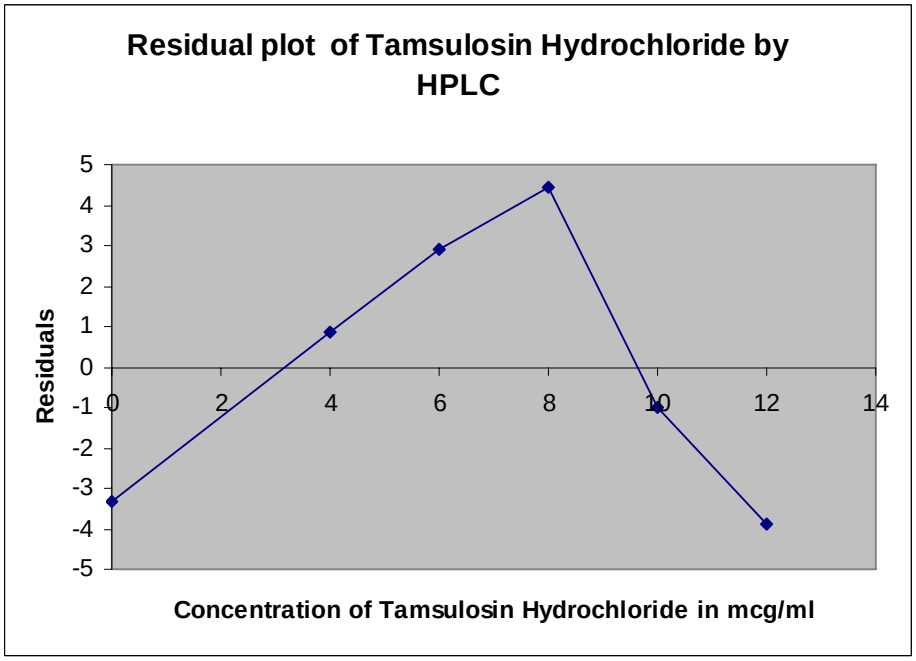
Graph No.1(b)



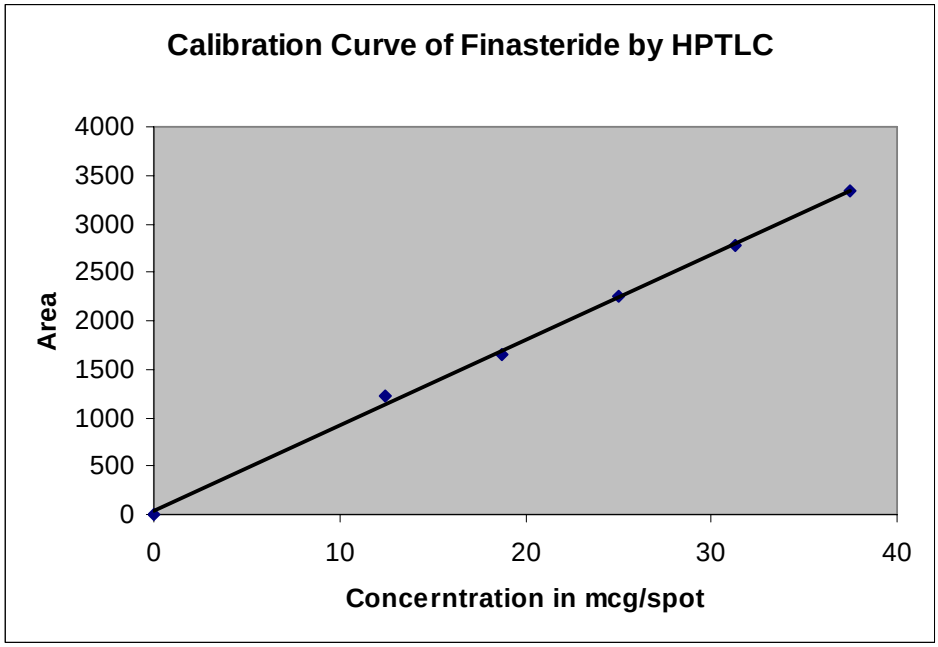
Graph No.1(c)



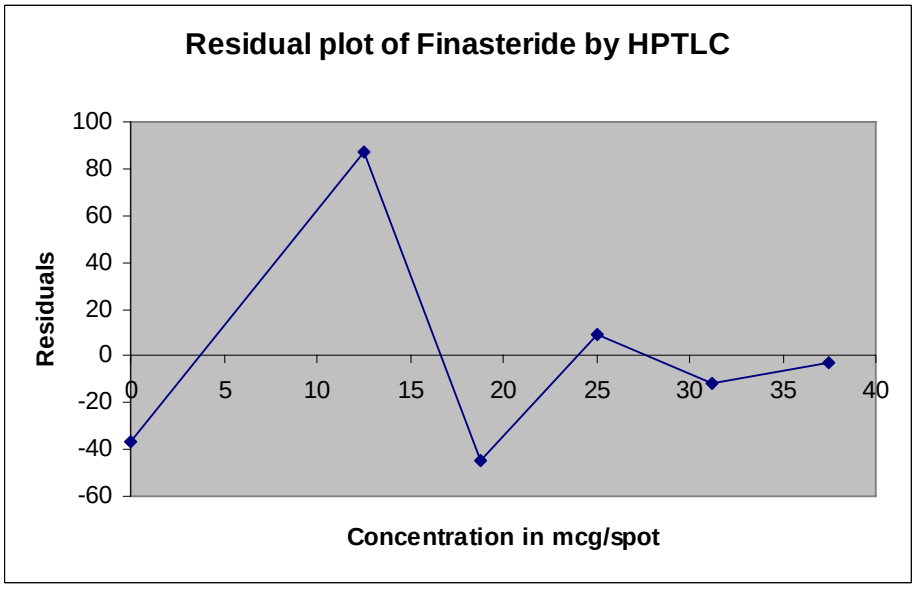
Graph No. 1(d)



Graph No. 2(a)



Graph No. 2(b)



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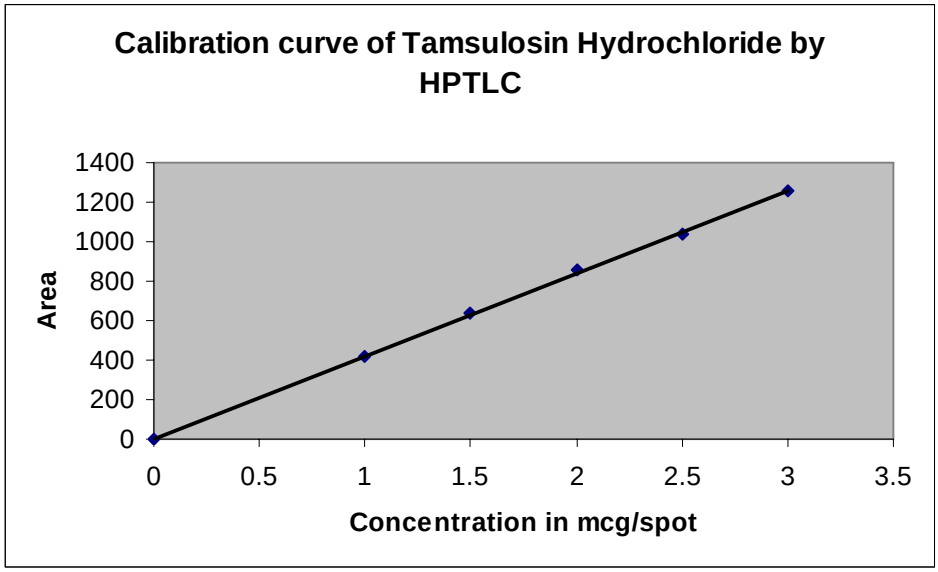
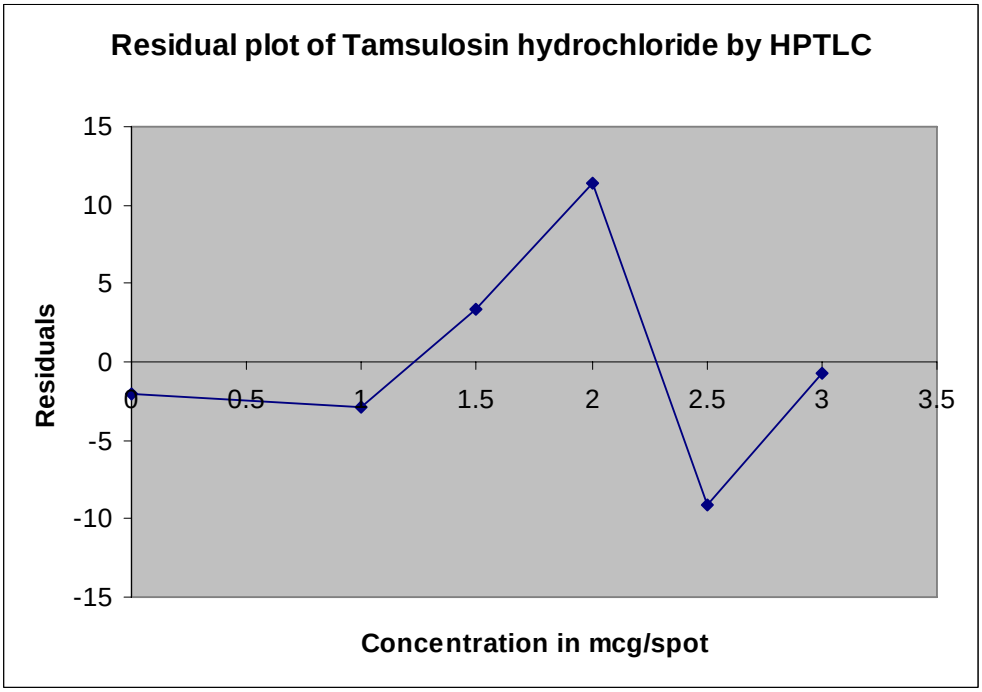
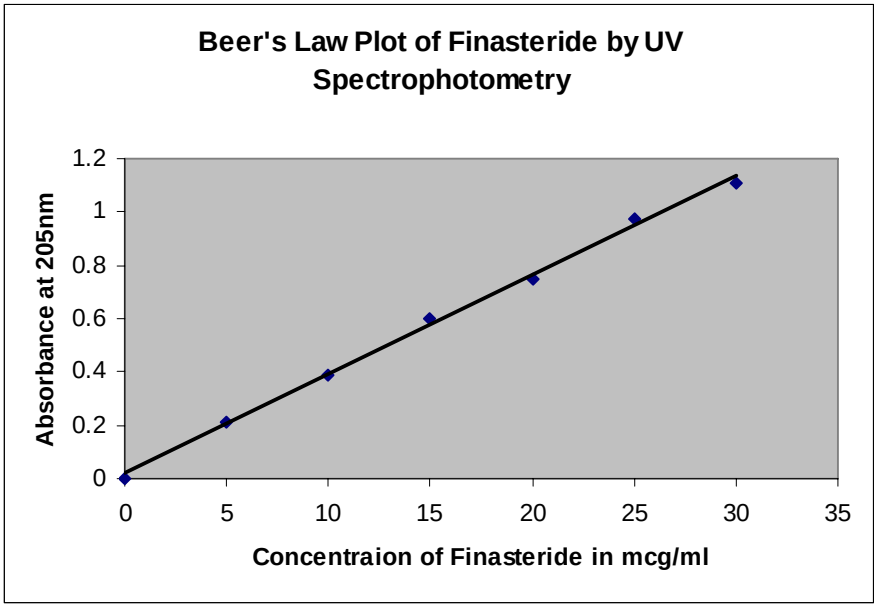


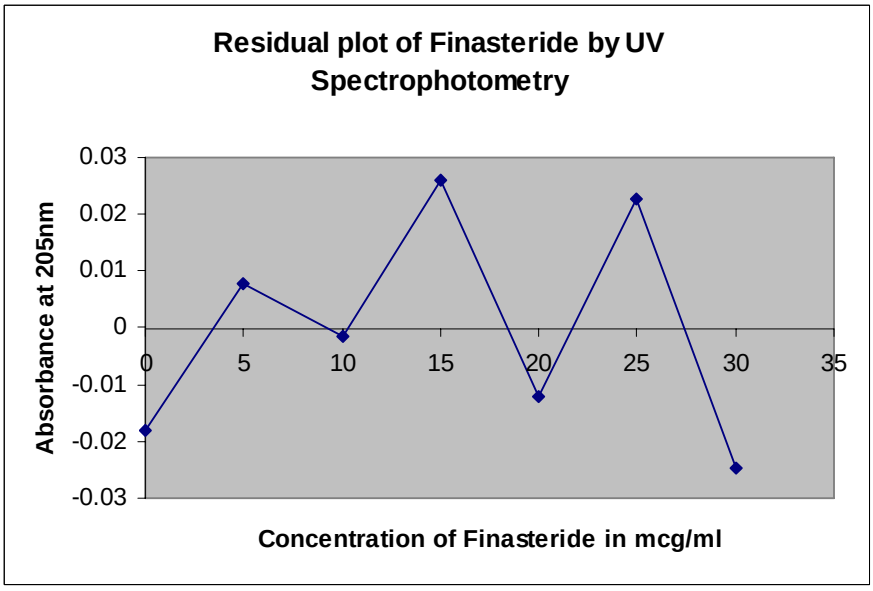
Graph No. 2(d)



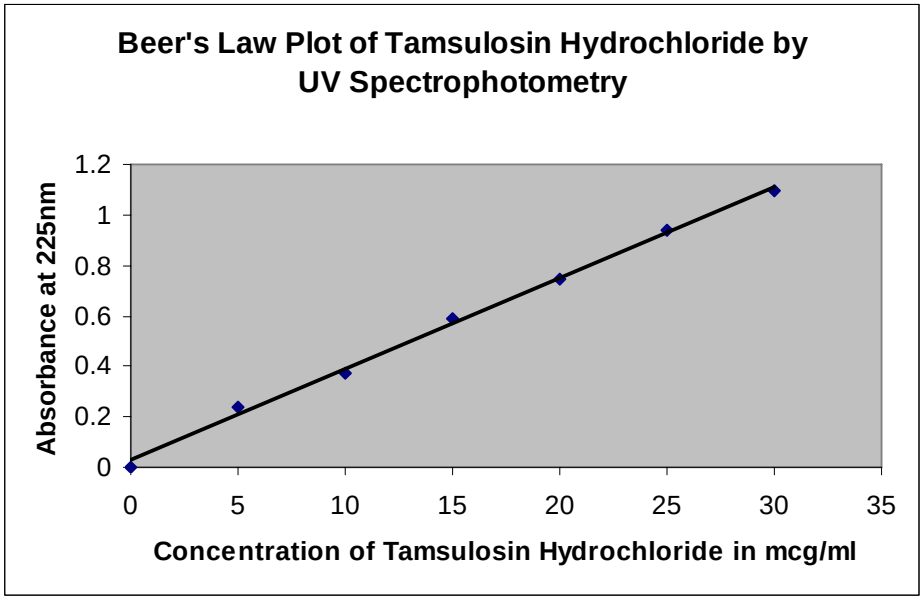
Graph No. 3(a)



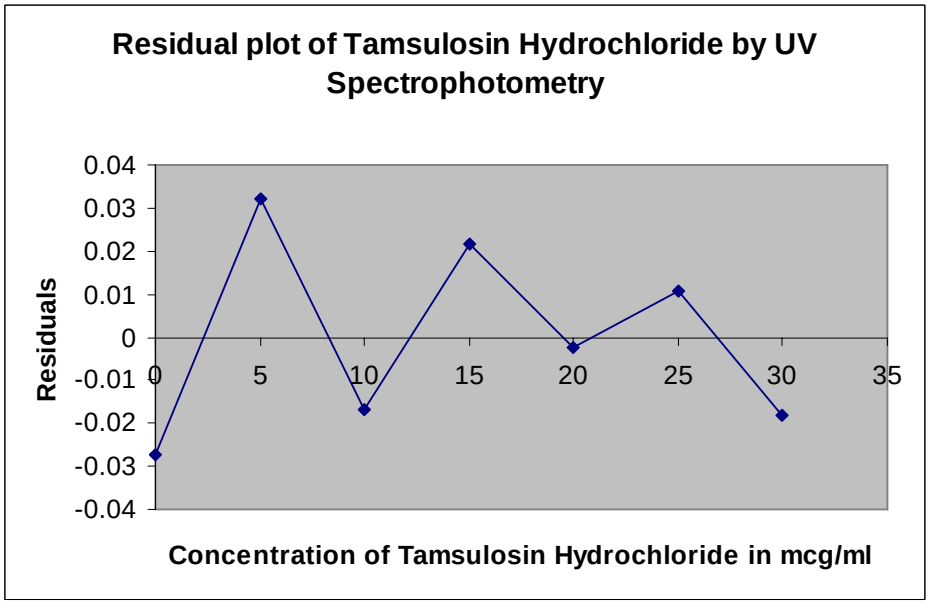
Graph No. 3(b)



Graph No. 3(c)

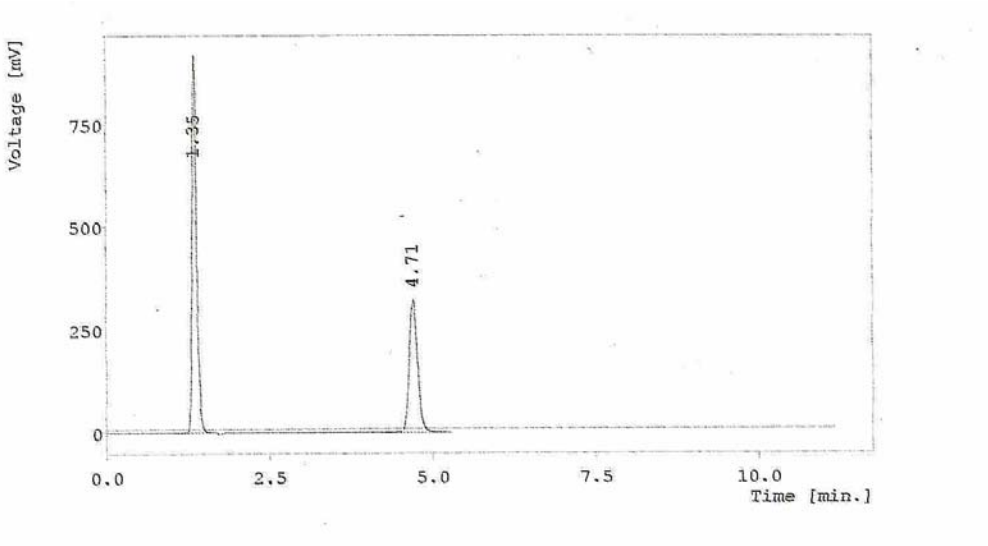


Graph No. 3(d)

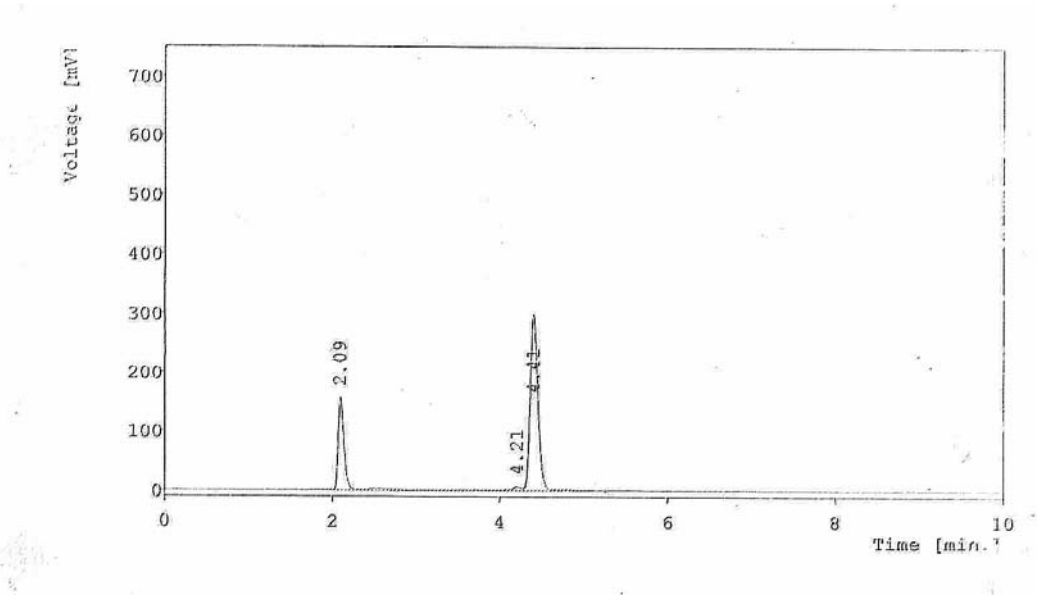


TRIALS PERFORMED IN HPLC METHOD DEVELOPMENT

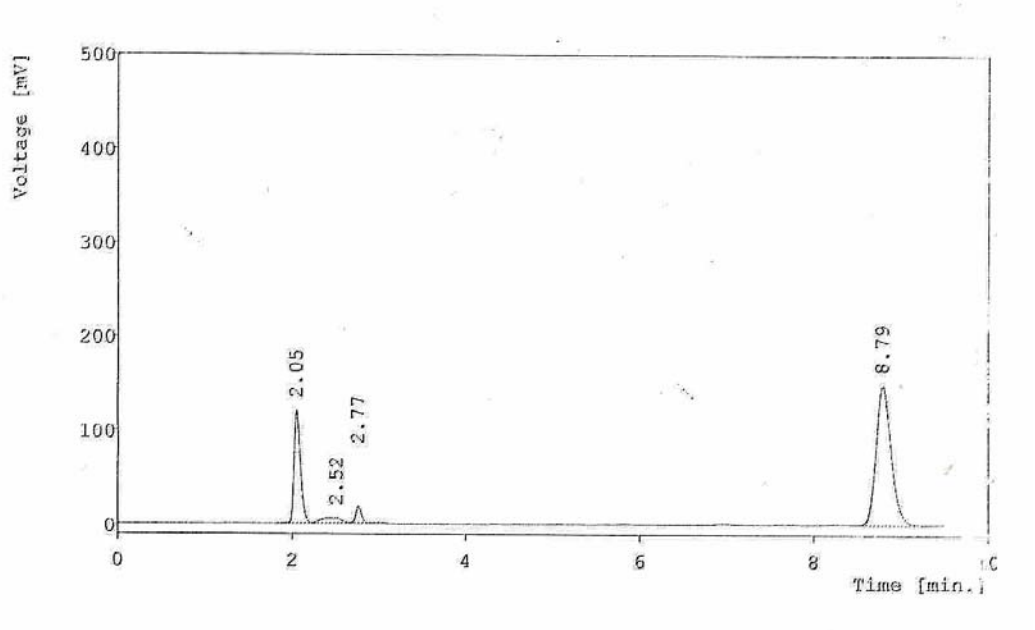
Chromatograph No. 1(a): Trial No.1



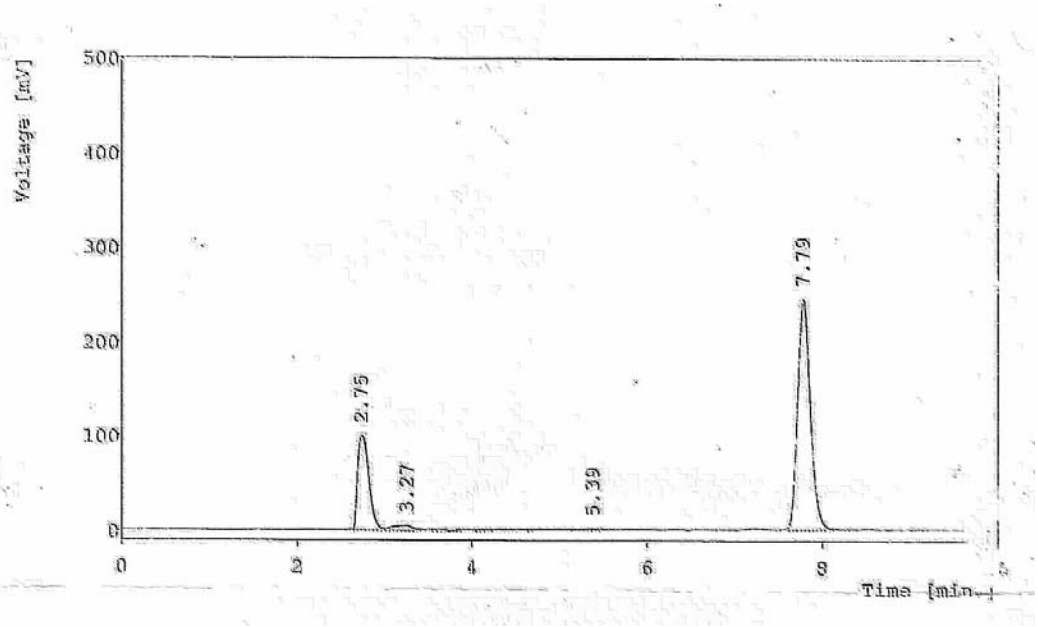
Chromatograph No. 1(b): Trial No: 2



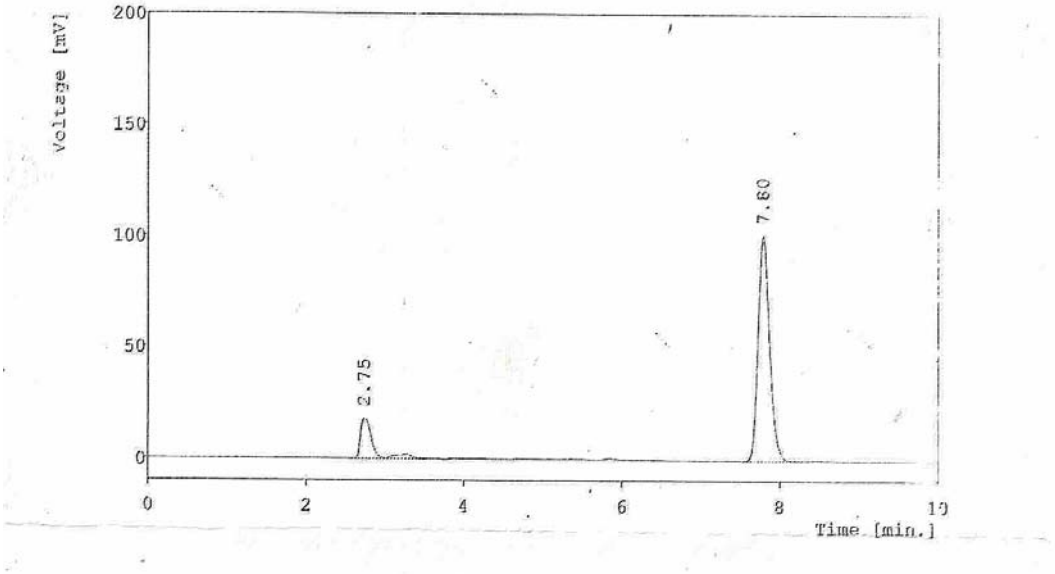
Chromatograph No. 1(c): Trial No:3



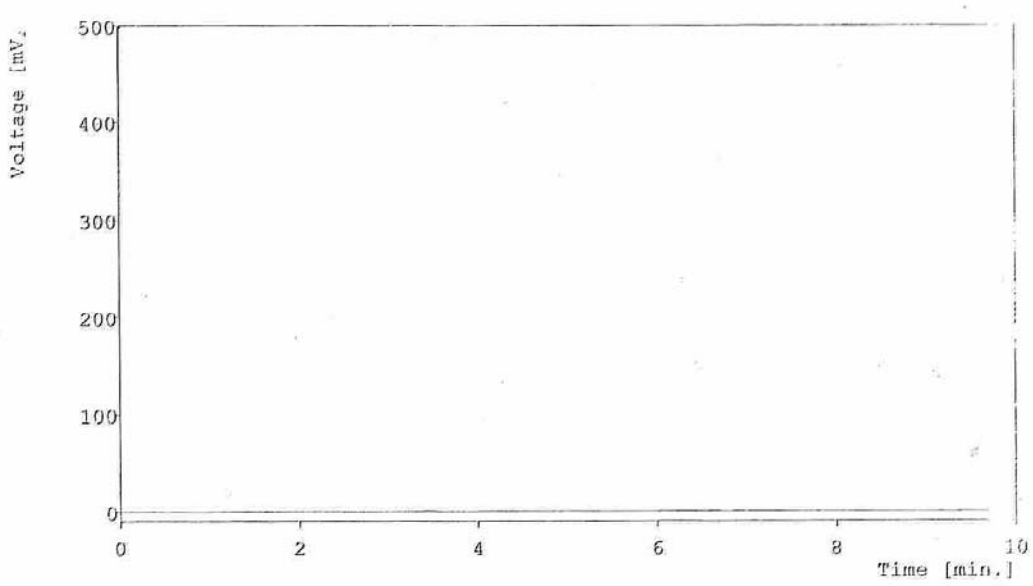
Chromatograph No. 1(d): Trial No:4



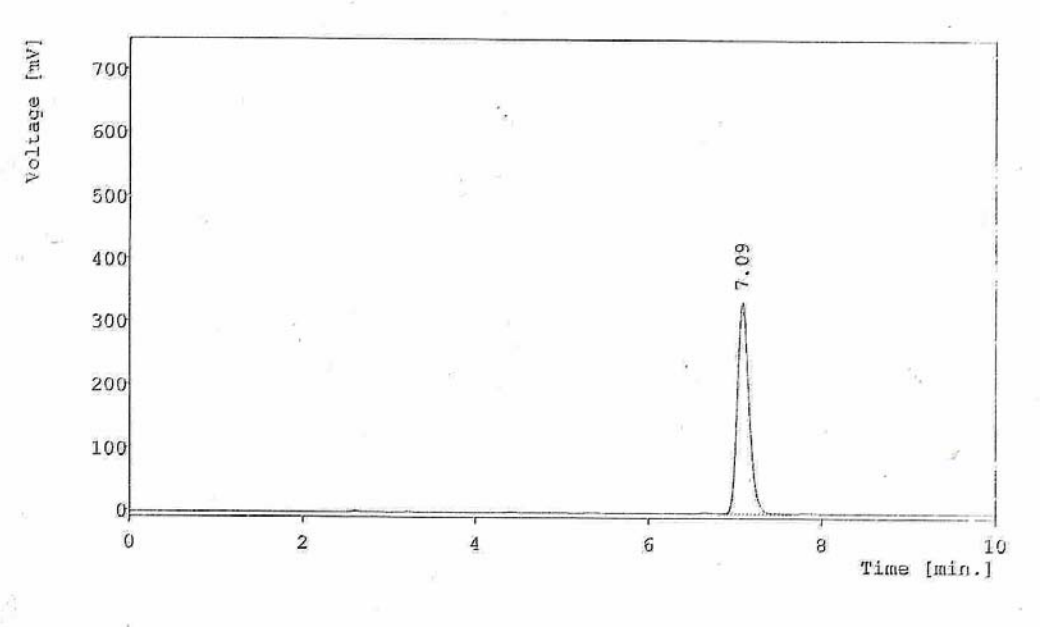
Chromatograph No. 1(e): Trial No.5



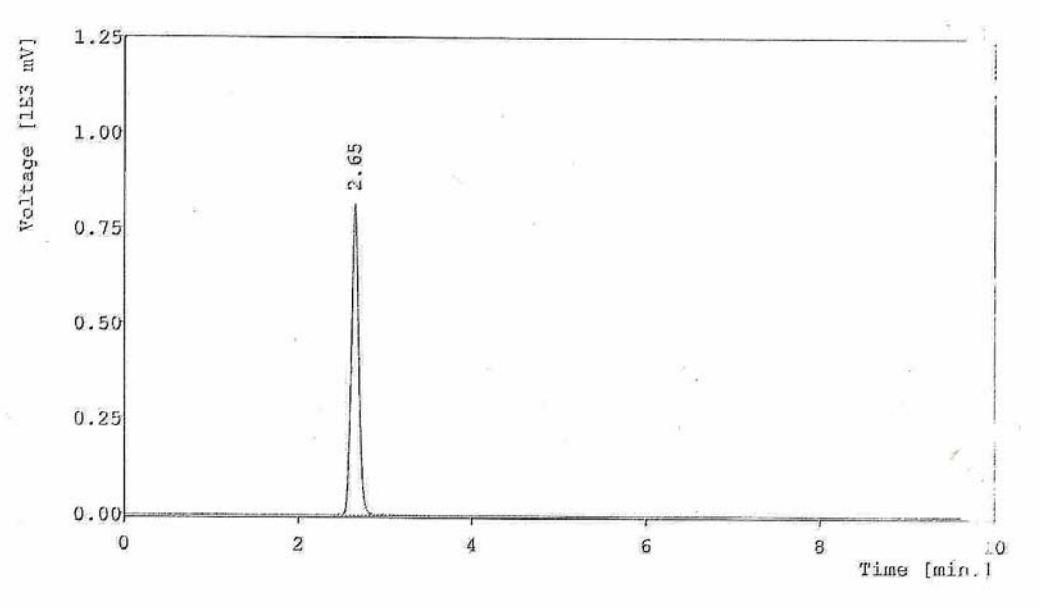
Chromatograph No. 1(f): Blank



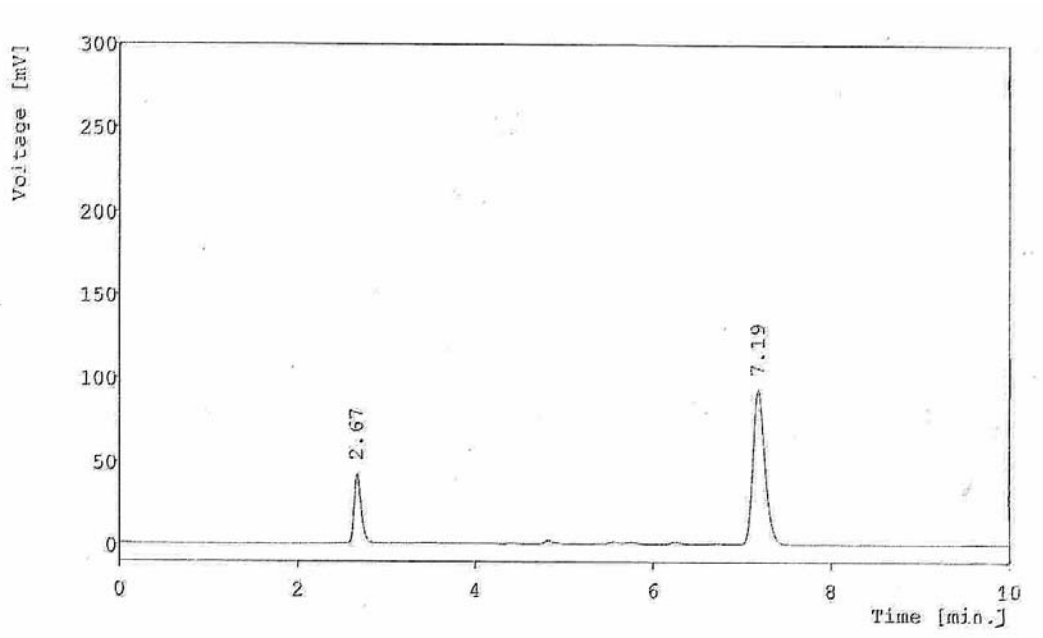
Chromatograph No. 1(g): Finasteride Standard



Chromatograph No. 1(h): Tamsulosin Hydrochloride Standard

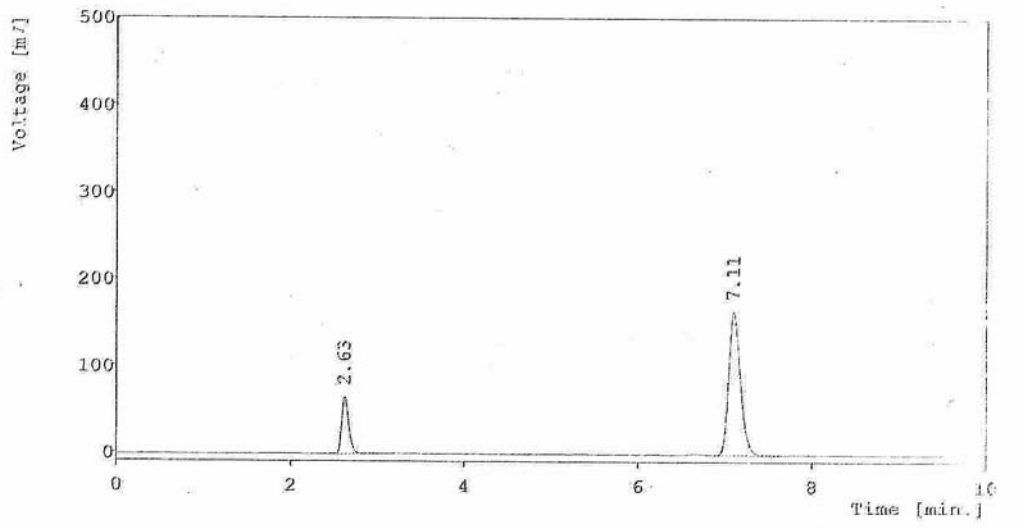


Chromatograph No. 1(i): Linearity Level 1



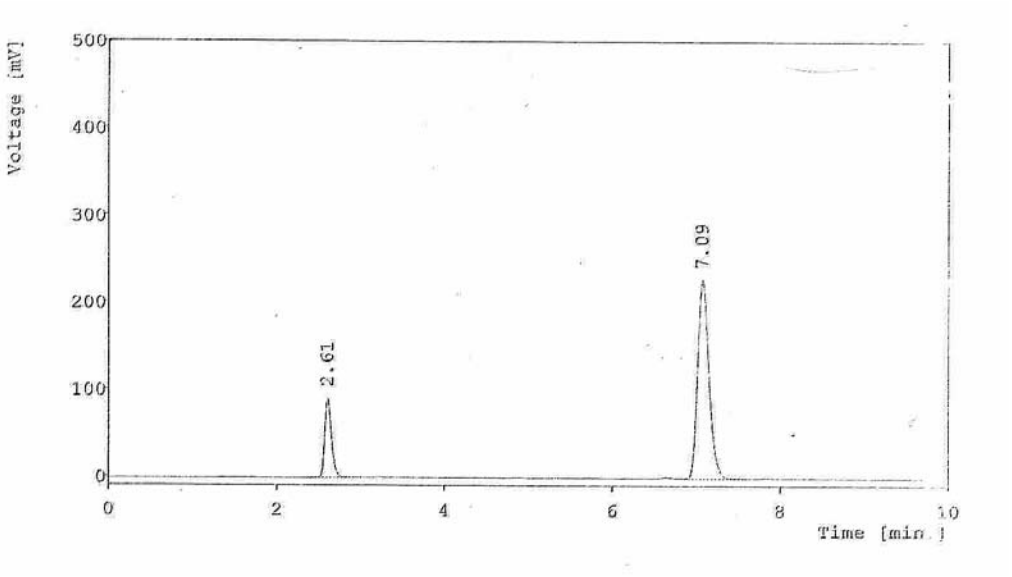
Peak No.	Reten. Time	Area [mV.s]	Height [mV]	W05 [min]	Area [%]	Height [%]
1	2.673	233.451	41.4825	0.0867	20.8908	30.8522
2	7.193	878.599	92.9729	0.1533	79.1092	69.1478
-	Total	1112.050	134.4554			

Chromatograph No. 1(j): Linearity Level 2



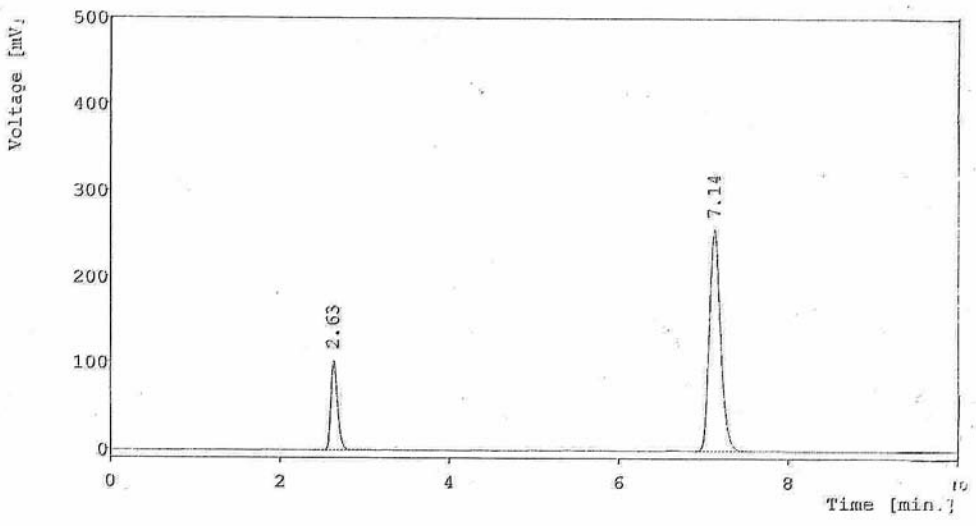
Peak No.	Reten. Time	Area [mV.s]	Height [mV]	W05 [min]	Area [%]	Height [%]
1	2.627	350.144	65.5247	0.0933	19.4441	28.3638
2	7.113	1442.121	165.4901	0.1533	80.5559	71.6362
-	Total	1792.265	231.0148			

Chromatograph No. 1(k): Linearity Level 3



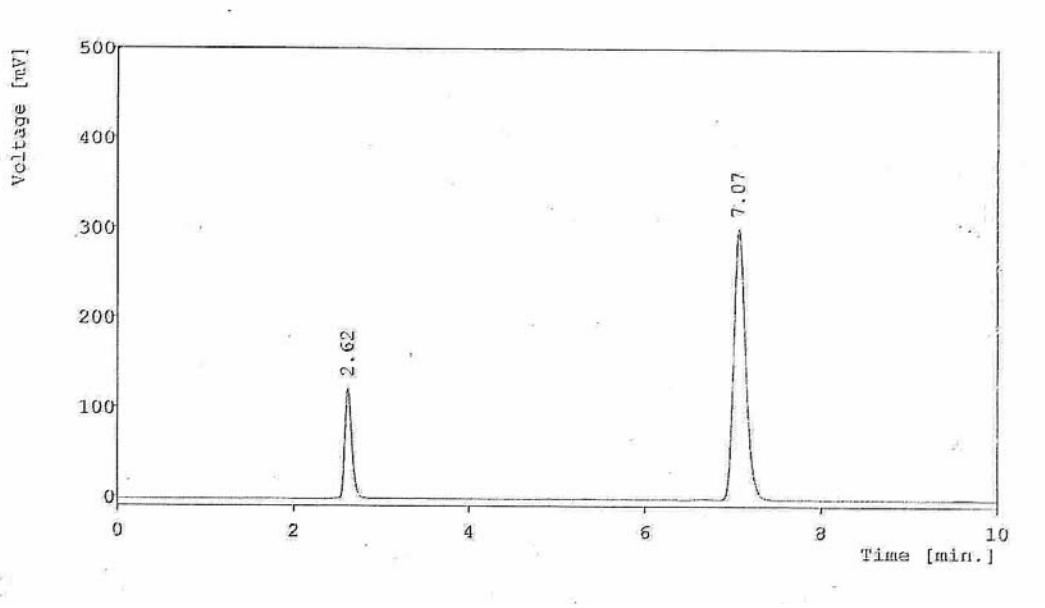
Peak No.	Reten. Time	Area [mV.s]	Height [mV]	W05 [min]	Area [%]	Height [%]
1	2.613	466.283	90.7033	0.0933	19.3153	28.4172
2	7.087	1801.245	228.4810	0.1467	80.8647	71.5848
-	Total	2267.528	319.1843			

Chromatograph No. 1(l): Linearity Level 4



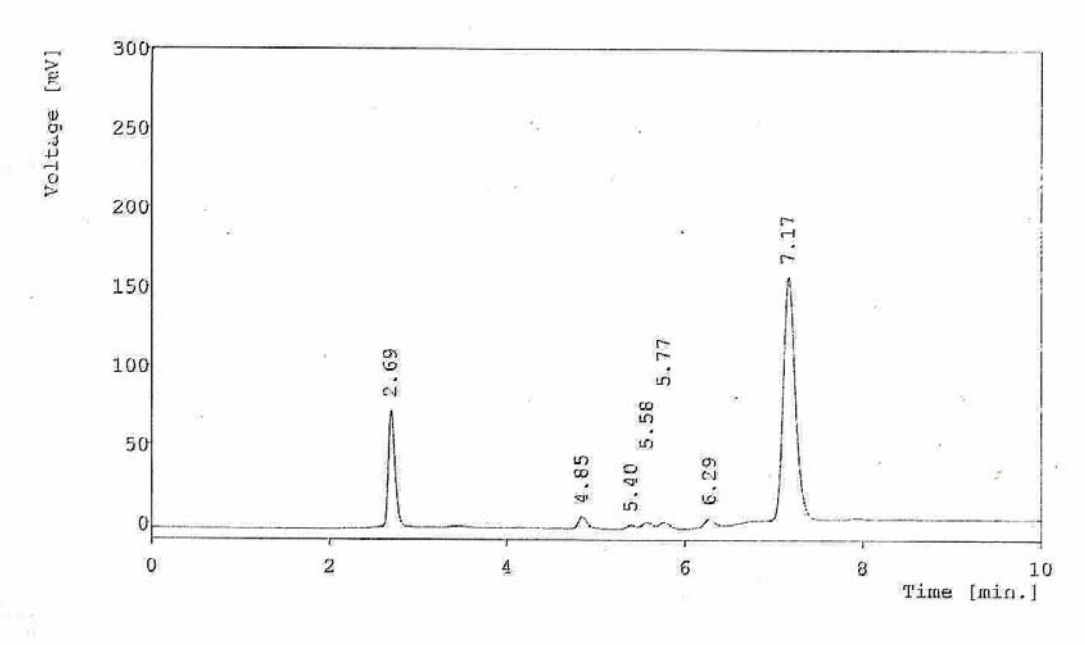
Peak No.	Reten. Time	Area [mV.s]	Height [mV]	W05 [min]	Area [%]	Height [%]
1	2.633	575.461	102.9119	0.0933	19.4840	28.5675
2	7.140	2401.502	257.3299	0.1533	80.5160	71.4325
-	Total	2976.963	360.2419			

Chromatograph No. 1(m): Linearity Level 5



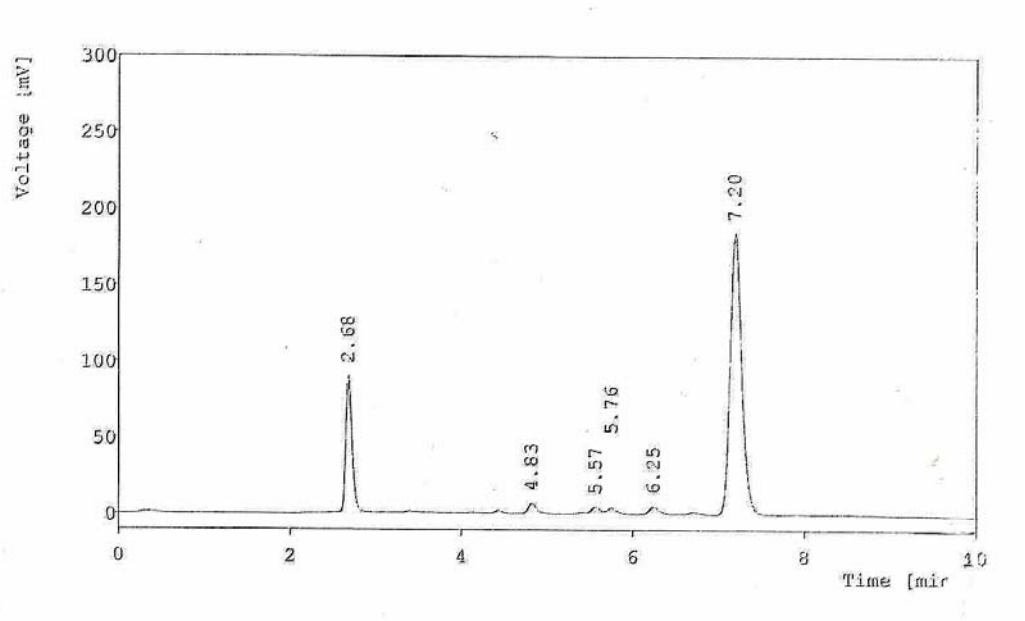
Peak No.	Reten. Time	Area [mV.s]	Height [mV]	W05 [min]	Area [%]	Height [%]
1	2.620	687.236	122.4690	0.0933	19.4673	28.8397
2	7.073	2715.651	302.1851	0.1533	80.5327	71.1603
-	Total	3402.887	424.6541			

Chromatograph No. 1(n): Sample



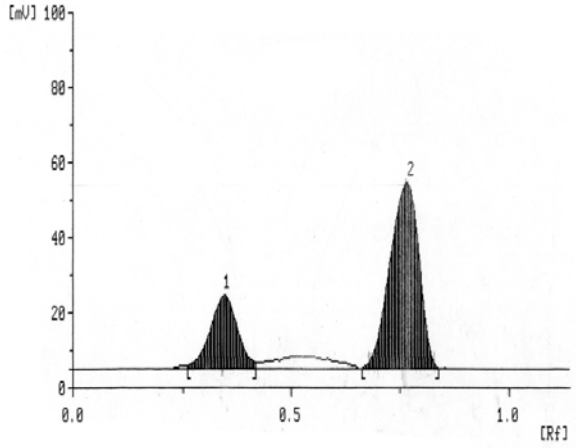
Peak No.	Reten. Time	Area [mV.s]	Height [mV]	W05 [min]	Area [%]	Height [%]
1	2.693	451.461	74.0847	0.0933	17.5183	28.9602
2	4.847	40.5736	7.6222	0.1067	2.2736	2.9796
3	5.400	10.9521	2.3996	0.1333	0.6770	0.9380
4	5.580	23.8283	4.3681	0.1333	1.4357	1.7075
5	5.773	26.6349	4.4225	0.1400	1.5548	1.7288
6	6.287	41.3507	5.8115	0.1433	2.1793	2.2718
7	7.173	1782.1679	157.3299	0.1533	74.3613	61.4141
-	Total	2376.9685	255.8152			

Chromatograph No. 1(o): Recovery Studies

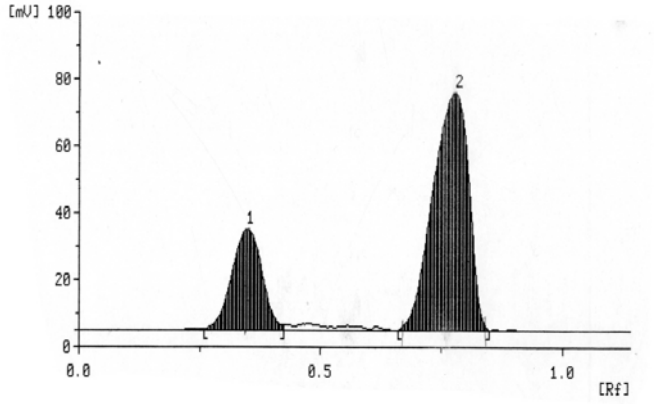


Peak No.	Reten. Time	Area [mV.s]	Height [mV]	W05 [min]	Area [%]	Height [%]
1	2.680	476.1102	89.9288	0.0867	20.1642	30.4449
2	4.827	48.4033	6.7695	0.1133	2.0202	2.2919
3	5.573	34.3325	4.7603	0.1333	1.8504	1.6117
4	5.760	21.7175	4.1037	0.1467	1.4908	1.3894
5	6.253	40.1599	5.0916	0.1400	1.8432	1.7239
7	7.200	1792.1679	184.7145	0.1467	72.6311	62.5382
-	Total	2412.8913	295.3624			

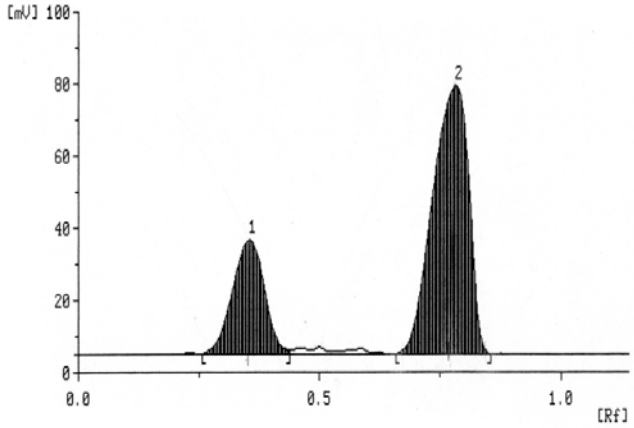
Chromatograph No. 2(a): Linearity Level 1



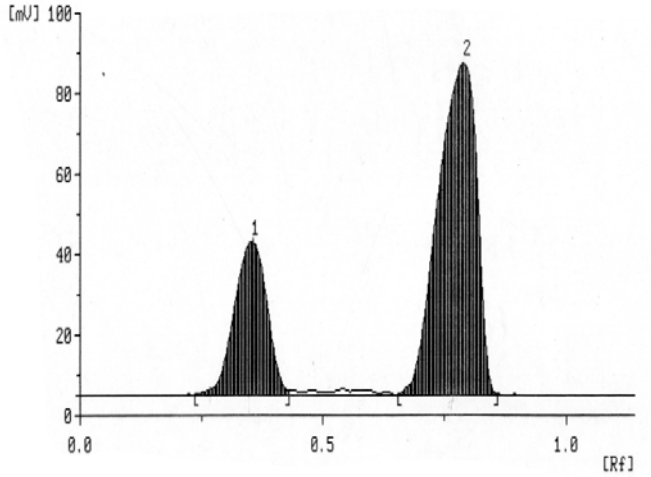
Chromatograph No. 2(b): Linearity Level 2



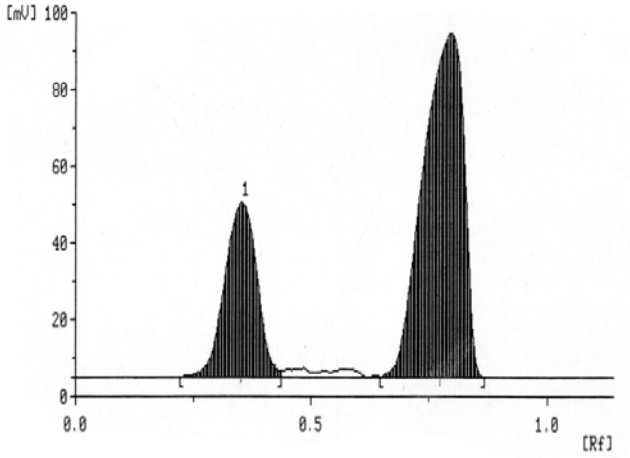
Chromatograph No. 2(c): Linearity Level 3



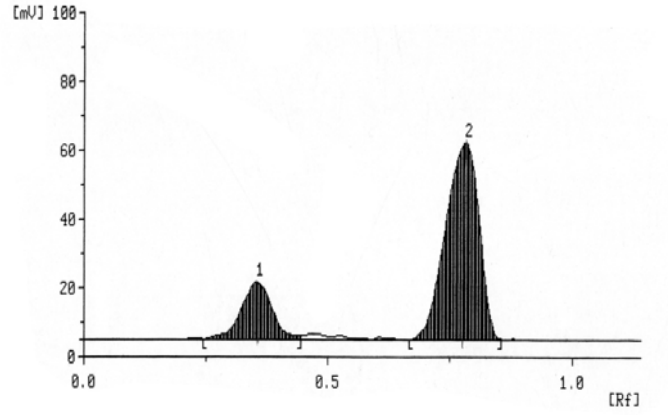
Chromatograph No. 2(d): Linearity Level 4



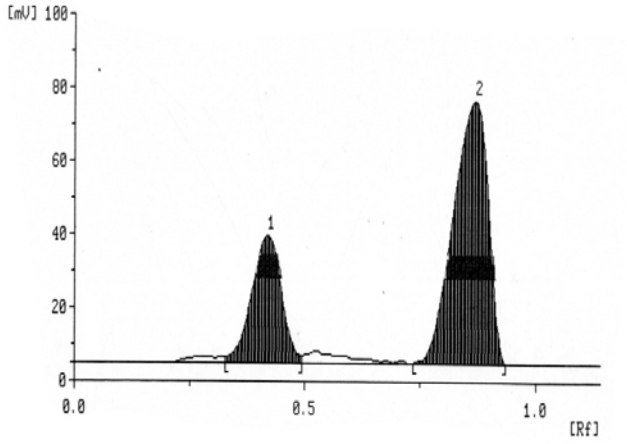
Chromatograph No. 2(e): Linearity Level 5



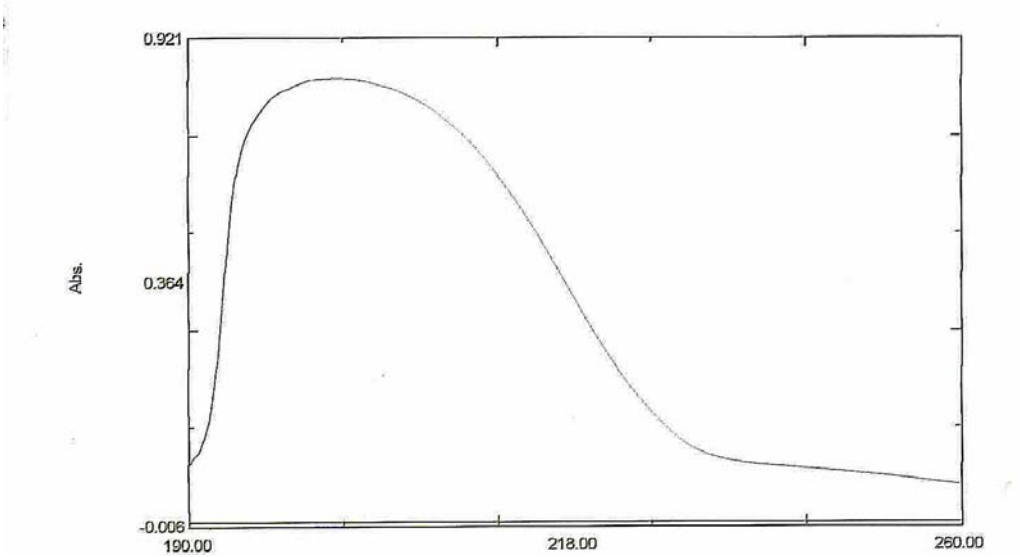
Chromatograph No. 2(f): Sample



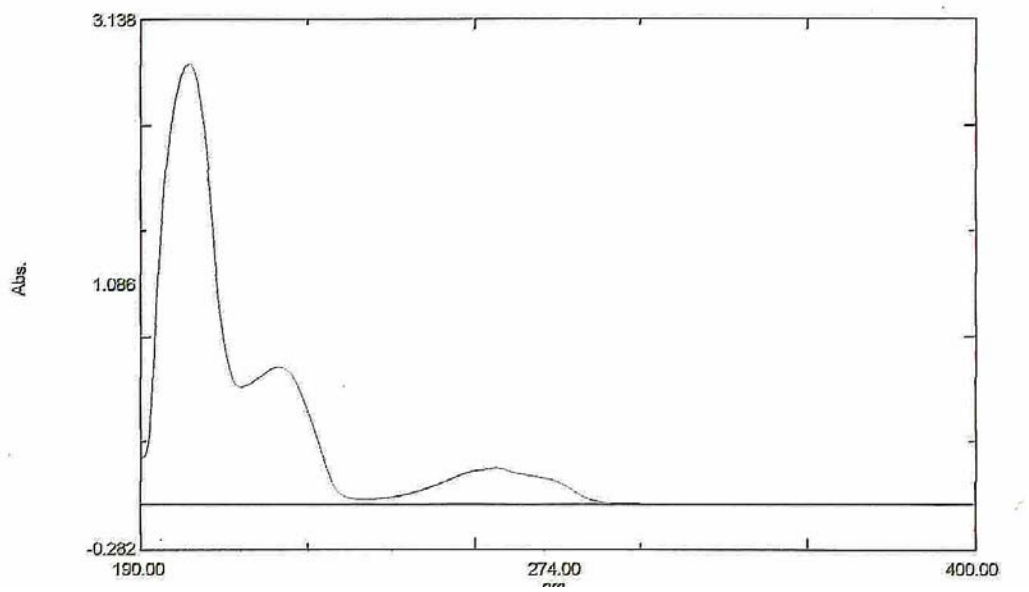
Chromatograph No. 2(f): Recovery Studies



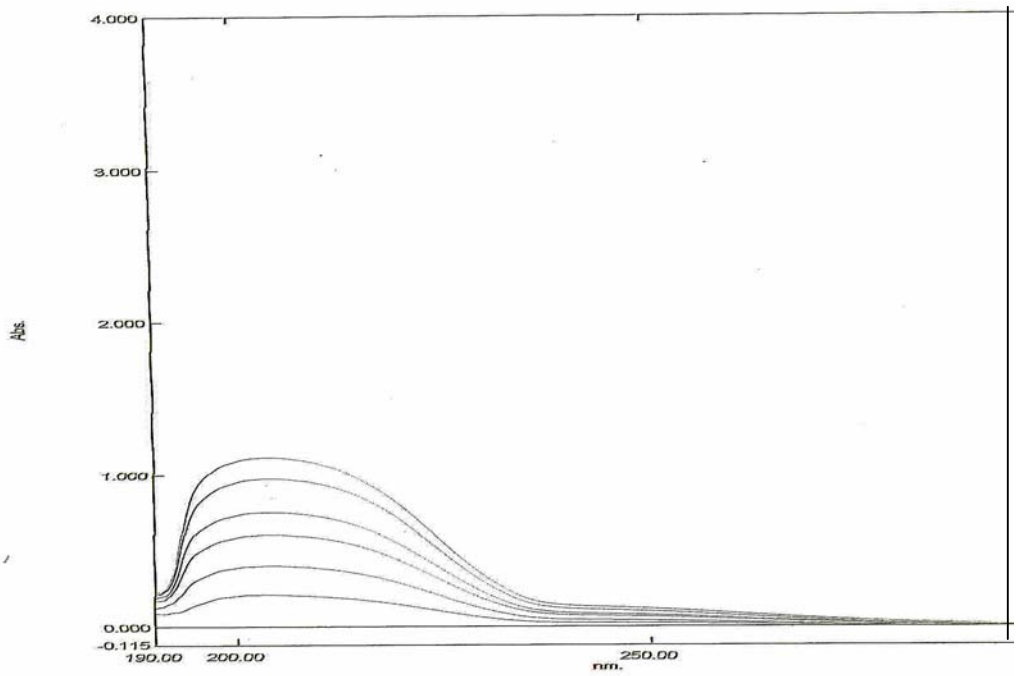
Spectrum No.1 (a): λ max of FINA by UV Spectrophotometry



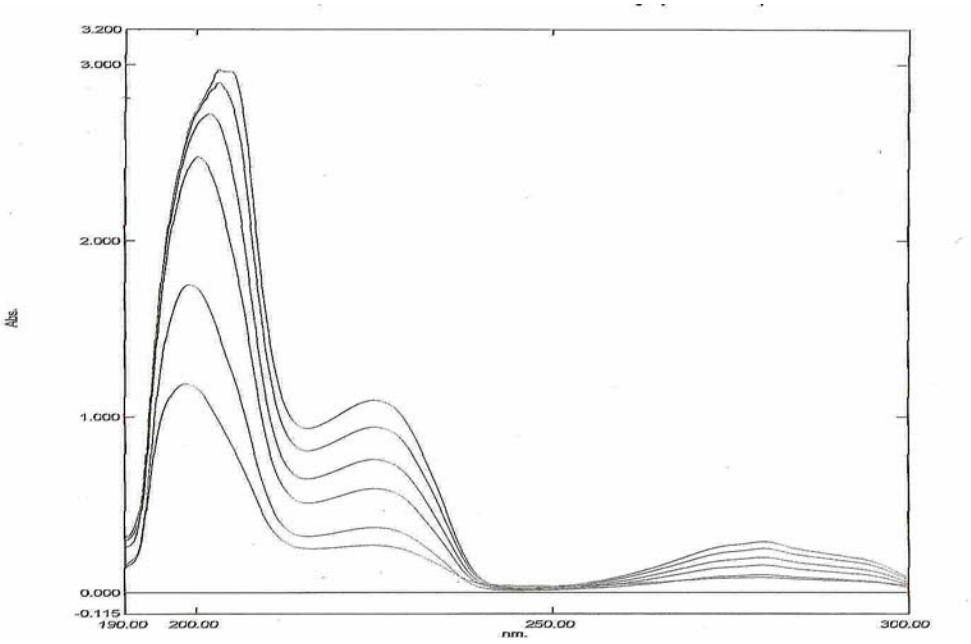
Spectrum No.1 (b): λ max of TAMS by UV Spectrophotometry



Spectrum No.1 (c): Overlay Spectrum Of FINA in concentration range obeying Beer's Law



Spectrum No.1 (d): Overlay Spectrum Of Tamsulosin Hydrochloride in concentration range obeying Beer's Law



Spectrum No.1 (e): Overlay Spectrum Of Finasteride And Tamsulosin Hydrochloride Showing Isoabsorptive Points